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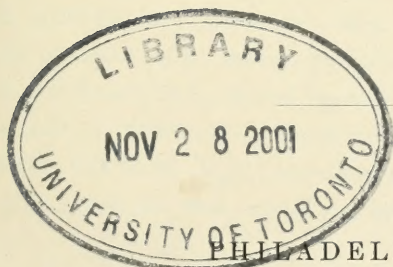
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PROCEEDINGS
OF THE
PATHOLOGICAL SOCIETY
OF PHILADELPHIA.

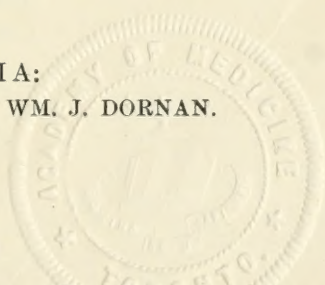
NEW SERIES, VOLUME XI.
OLD SERIES, VOLUME XXIX.

*CONTAINING THE TRANSACTIONS OF THE SOCIETY FROM
OCTOBER, 1907, TO OCTOBER, 1908.*

EDITED BY
FRED H. KLAER, M.D.,
RECORDER OF THE SOCIETY.



PHILADELPHIA:
PRINTED FOR THE SOCIETY BY WM. J. DORNAN.
1908.



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RENÉ LA ROCHE, M.D., elected 1858.

ALFRED STILLÉ, M.D., LL.D., elected 1859, 1861, and 1862.

EDWARD HARTSHORNE, M.D., elected 1860 and 1863.

J. M. DA COSTA, M.D., LL.D., elected 1864, 1865, and 1866.

JOHN H. PACKARD, M.D., elected 1867 and 1868.

S. WEIR MITCHELL, M.D., LL.D., elected 1869.

JOHN ASHHURST, JR., M.D., LL.D., elected 1870.

JAMES H. HUTCHINSON, M.D., elected 1871 and 1872.

WILLIAM PEPPER, M.D., LL.D., elected 1873, 1874, and 1875.

H. LENOX HODGE, M.D., elected 1876.

S. W. GROSS, M.D., elected 1879.

JAMES TYSON, M.D., elected 1882 and 1883.

E. O. SHAKESPEARE, M.D., elected 1884.

J. C. WILSON, M.D., elected 1885 and 1886.

F. P. HENRY, M.D., elected 1887 and 1888.

HENRY F. FORMAD, M.D., elected 1889 and 1890.

ARTHUR V. MEIGS, M.D., elected 1891 and 1892.

J. H. MUSSER, M.D., elected 1893, 1894, 1895, and 1896.

W. E. HUGHES, M.D., elected 1897 and 1898.

F. A. PACKARD, M.D., elected 1899 and 1900.

CHARLES W. BURR, M.D., elected 1901 and 1902.

ALFRED STENGEL, M.D., elected 1903 and 1904.

W. M. L. COPLIN, M.D., elected 1905 and 1906.

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OF THE
PATHOLOGICAL SOCIETY OF PHILADELPHIA.

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LIST OF MEMBERS.

ORIGINAL MEMBERS.

JAMES DARRACH, 5923 Greene Street, Germantown.

S. WEIR MITCHELL, 1524 Walnut Street.

GEORGE C. HARLAN, 1700 Walnut Street.

MEMBERS.

ELECTED

1894 Abbott, A. C., 4229 Baltimore Avenue.

1907 Addison, W. H., 3928 Pine Street.

1899 Adler, L. H., Jr., 1610 Arch Street.

1907 Allen, Alfred Reginald, 1111 South Twenty-first Street.

1898 Allen, L. M., 3100 Wharton Street.

1906 Allyn, Herman B., 501 South Forty-second Street.

1889 Anders, J. M., 1605 Walnut Street.

1901 Anspach, B. M., 119 South Twentieth Street.

1906 d'Apéry, Tello J., 767 North Fortieth Street.

1900 Artelt, H., 1521 North Eighth Street.

1891 Ashton, Thomas G., 1814 South Rittenhouse Square.

1895 Ashton, William E., 2011 Walnut Street.

1897 Babcock, W. Wayne, 3302 North Broad Street.

1887 Baker, G. F., 1818 Spruce Street.

1906 Beardsley, E. J. G., 2030 Chestnut Street.

1885 Beates, Henry, Jr., 260 South Sixteenth Street.

1900 Behrend, Moses, 1331 North Franklin Street.

1871 Bennett, Wm. H., 1837 Chestnut Street.

1887 Berens, Bernard, 2041 Chestnut Street.

1900 Bergey, D. H., 3965 Brown Street.

1905 Bernheim, Albert, 1411 Spruce Street.

1908 Bethel, John P., 1825 Fairmount Avenue.

1905 Blackwood, J. D., 5346 Wayne Avenue, Germantown.

ELECTED

- 1905 Bland, P. B., 1840 South Broad Street.
1893 Bochroch, Max H., 937 North Eighth Street.
1892 Boger, John A., 2213 North Broad Street.
1908 Boice, J. Morton, 1700 Walnut Street.
1898 Boston, L. Napoleon, 1531 South Broad Street.
1893 Boyer, H. P., 4602 Baltimore Avenue.
1898 Brinton, Ward, 1423 Spruce Street.
1900 Brown, H. MacV., 4608 Baltimore Avenue.
1895 Brubaker, A. P., 105 North Thirty-fourth Street.
1893 Bryan, J. Roberts, 4200 Chestnut Street.
1901 Buckley, A. C., 1705 North Fifteenth Street.
1887 Burr, Charles W., 1327 Spruce Street.
1908 Busch, John William, 1634 South Broad Street.
- 1905 Cadbury, W., 4044 Chestnut Street.
1907 Cadwalader, W. B., 1710 Locust Street.
1906 Campbell, J. M., 655 North Twelfth Street.
1902 Carncross, H. L., 721 Pine Street.
1903 Carnett, J. B., 318 South Fifteenth Street.
1893 Carpenter, H. B., 1805 Spruce Street.
1902 Carpenter, H. C., 1805 Spruce Street.
1897 Carpenter, J. T., Jr., 1624 Walnut Street.
1887 Cattell, H. W., 3709 Spruce Street.
1906 Chandler, Swithin, 2010 Chestnut Street.
1898 Chestnut, J. E., 1817 Frankford Avenue.
1899 Clark, John G., 2017 Walnut Street.
1866 Cleemann, Richard A., 2135 Spruce Street.
1908 Cohen, A. J., 738 Pine Street.
1872 Cohen, J. Solis, 1824 Chestnut Street.
1902 Cohen, M. Solis, 4110 Parkside Avenue.
1883 Cohen, S. Solis, 1525 Walnut Street.
1896 Coley, Thomas Luther, 257 South Twenty-first Street.
1907 Conaway, W. P., 1723 Pacific Avenue, Atlantic City, N. J.
1905 Cope, Thomas A., 6504 Germantown Avenue.
1890 Coplin, W. M. L., 1529 South Broad Street.
1901 Craig, F. A., 732 Pine Street.
1906 Crispin, E. L., Pennsylvania Hospital.
1899 Cruice, John M., 1815 Spruce Street.
1901 Cryer, M. H., 1420 Chestnut Street.
1904 Cummins, W. T., 226 South Fifty-third Street.
1898 Currie, Thos. R., 113 East Cumberland Street.
1871 Curtin, Roland G., 22 South Eighteenth Street.

ELECTED

- 1896 Da Costa, J. C., Jr., 1022 Spruce Street.
 1894 Da Costa, J. Chalmers, 2045 Walnut Street.
 1885 Daland, Judson, 317 South Eighteenth Street.
 1857 Darrach, James, 5923 Greene Street, Germantown (O. M.).
 1882 Davis, G. G., 1814 Spruce Street.
 1893 Davisson, Alex. H., Ardmore, Pa.
 1900 Deal, J. C., 5301 Haverford Avenue.
 1889 Deaver, H. C., 1534 North Fifteenth Street.
 1885 Deaver, J. B., 1634 Walnut Street.
 1882 Dercum, F. X., 1719 Walnut Street.
 1906 Despard, D. L., 1900 Chestnut Street.
 1904 Dever, Francis T., 275 South Fifty-seventh Street.
 1907 Dintenfass, 415 Pine Street.
 1905 Doland, Charles M., Pennsylvania Hospital.
 1907 Donnhauser, J. L., Pennsylvania Hospital.
 1902 Dorrance, G. M., 1716 Locust Street.
 1894 Dougherty, S. W., 256 South Sixteenth Street.
 1889 Downs, Norton, 215 West Walnut Lane, Germantown.
 1903 Drein, W. C., 1438 North Fifteenth Street.
 1866 Duer, Edward L., 1606 Locust Street.
 1907 Dugan, W. J., 2224 South Broad Street.
 1876 Dulles, C. W., 4101 Walnut Street.
 1900 Dye, F. H., 1830 Girard Avenue.

 1896 Edsall, David L., 1432 Pine Street.
 1902 Ellis, A. G., 2524 North Seventeenth Street.
 1900 Erck, T. A., 251 South Thirteenth Street.
 1891 Eshner, A. A., 1019 Spruce Street.
 1906 Evans, Clarke, 1900 Chestnut Street.
 1902 Evans, J. S., 2014 Locust Street.

 1901 Farr, C. B., 211 South Seventeenth Street.
 1881 Fenton, T. H., 1319 Spruce Street.
 1907 Fetterolf, George, 330 South Sixteenth Street.
 1903 Fife, C. A., 318 South Fifteenth Street.
 1876 Fisher, Henry M., 917 Pine Street.
 1907 Fleisher, M. S., 6357 Sherwood Road, Overbrook, Pa.
 1907 Flick, Lawrence F., 738 Pine Street.
 1908 Foster, G. B., Philadelphia Hospital.
 1903 Fox, H., 4443 Spruce Street.
 Fox, J. M., Torresdale, Pa.
 1894 Fox, L. Webster, 1304 Walnut Street.
 1899 Francine, A. P., 218 South Fifteenth Street.

ELECTED

- 1895 Frazier, Charles Harrison, 1724 Spruce Street.
1890 Friebeis, George, 1906 Chestnut Street.
1903 Funke, J., 1130 Spruce Street.
1885 Fussell, M. H., 189 Green Lane, Manayunk.
- 1902 Geisler, Howard D., 202 High Street, Germantown.
1870 Getchell, Frank H., 1432 Spruce Street.
1905 Gilbride, John J., 2412 North Sixth Street.
1902 Gildersleeve, Nathaniel, Laboratory of Hygiene, University of Pennsylvania.
1902 Gilliland, S. H., Marietta, Pa.
1908 Ginsburg, Nate, 340 South Fifteenth Street.
1894 Girvin, John H., 3924 Walnut Street.
1902 Githens, T. S., 1337 Pine Street.
1898 Gittings, J. Claxton, 3942 Chestnut Street.
1899 Given, E. E. W., 2714 Columbia Avenue.
1882 Godey, H. E., N. E. corner Nineteenth and Spruce Streets.
1900 Goepp, R. M., 332 South Fifteenth Street.
1905 Goldberg, H. G., 1733 Chestnut Street.
1906 Goodman, E. H., 2035 Chestnut Street.
1905 Gordon, Alfred, 1430 Pine Street.
1902 Graham, E. E., 1713 Spruce Street.
1905 Gray, R. L., 3031 North Broad Street.
1890 Grayson, C. P., 251 South Sixteenth Street.
1883 Griffith, J. P. C., 1810 Spruce Street.
1897 Gross, Wm. D., 701 North Fortieth Street.
1906 Guilfoyle, W. F., 3722 Walnut Street.
1890 Gummey, Frank B., 5418 Greene Street, Germantown.
1900 Gwyn, N. B., 23 South Twenty-first Street.
- 1893 Hamill, S. M., 1822 Spruce Street.
1893 Hand, Alfred, Jr., 1724 Pine Street.
1885 Hare, Hobart Amory, N. W. cor. Eighteenth and Spruce Streets.
1857 Harlan, G. C., 1700 Walnut Street (O. M.).
1890 Hartzell, M. B., 3644 Chestnut Street.
1904 Hatfield, C. J., 2008 Walnut Street.
1901 Hawke, W. W., Philadelphia Hospital.
1900 Head, J., 1500 Locust Street.
1870 Henry, Frederick P., 1635 Locust Street.
1896 Henry, John N., 252 South Sixteenth Street.
1903 Herzberg, M., 5355 Webster Street.
1880 Hewson, Addinell, Jr., 2120 Spruce Street.
1903 Hill, H. K., 1706 Locust Street.

ELECTED

- 1908 Hindman, S. S., Glenolden, Pa.
1899 Hitchens, A. P., Glenolden, Pa.
1899 Holloway, T. B., 1819 Chestnut Street.
1905 Holmes, E. B., 2030 Chestnut Street.
1905 Horne, S. Hamill, 1433 Walnut Street.
1905 Hosmer, C. M., 2040 Chestnut Street.
1904 Hoyt, D. M., 3604 Chestnut Street.
1882 Hughes, W. E., Fortieth and Chestnut Streets.
1904 Hume, J. E., 900 South Forty-ninth Street.
1901 Hunsicker, C. H., 1614 North Broad Street.
1902 Hunter, John W., 1934 Chestnut Street.
- 1901 Irwin, J. W., 1923 Vine Street.
- 1904 Jenks, Horace H., 920 Clinton Street.
1908 Johnson, Lucius W., Philadelphia Hospital.
1895 Jopson, J. H., 1824 Pine Street.
1898 Judson, Chas. F., 1539 Pine Street.
1899 Jump, H. D., 4634 Chester Avenue.
- 1898 Kalteyer, F. J., 214 South Fifteenth Street.
1901 Kane, J. A. B., 211 South Seventeenth Street.
1908 Karsner, Howard T., 1320 South Broad Street.
1906 Keene, Floyd E., 334 South Sixteenth Street.
1895 Kelly, A. O. J., 1911 Pine Street.
1906 Kelly, James A., 1612 North Seventeenth Street.
1905 Kelly, Thos. C., 128 East Price Street, Germantown.
1908 Kelsey, Ernest W., 1217 Spruce Street.
1899 Kennedy, L. F., 301 Mauch Chunk Street, Pottsville, Pa.
1888 Kirby, Ellwood R., 1202 Spruce Street.
1905 Klaer, F. H., 334 South Sixteenth Street.
1900 Klapp, W. P., 1716 Spruce Street.
1898 Knipe, J. C., 2035 Chestnut Street.
1901 Kohn, B., 1325 North Thirteenth Street.
1908 Kotz, Adam L., Easton, Pa.
1908 Krumbhaar, Ed. B., Pennsylvania Hospital.
1895 Krusen, Wilmer, 127 North Twentieth Street.
1892 Kyle, D. Braden, 1517 Walnut Street.
- 1899 Landis, H. R. M., 130 South Twenty-third Street.
1890 Laplace, Ernest, 1828 South Rittenhouse Square.
1905 Lavenson, Ralph S., 1218 Locust Street.
1894 Leach, W. W., Eastern State Penitentiary, Medical Department.

ELECTED

- 1869 Leaman, Henry, 828 North Broad Street.
 1888 Leidy, Joseph, 1319 Locust Street.
 1905 L'Engle, Edward M., 132 South Twenty-third Street.
 1887 Leopold, Isaac, 1520 Franklin Street.
 1904 Leopold, S., 1632 Franklin Street.
 1875 Lewis, Morris J., 1316 Locust Street.
 1894 Lincoln, C. W., 314 E. Lancaster Avenue, St. David's, Pa.
 1899 Lindauer, Eugene, 2018 North Thirty-second Street.
 1886 Lloyd, James Hendrie, 3918 Walnut Street.
 1898 Lodholtz, Edward, 3103 Diamond Street.
 1903 Loeb, Leo, University of Pennsylvania.
 1894 Loeb, Ludwig, 1421 North Fifteenth Street.
 1901 Longcope, W. T., 323 South Sixteenth Street.
 1875 Longstreth, Morris, 1416 Spruce Street.
 Ludlum, S. D., Friends' Asylum, Frankford.
 1903 Lukens, G. T., Fifth Ave. and Fayette St., Conshohocken, Pa.

 1896 McCarthy, D. J., 1329 Spruce Street.
 1904 McClary, Samuel, 3d, 308 South Fifty-second Street.
 1880 McClellan, George, 1116 Spruce Street.
 1892 McFarland, J., 442 W. Stafford Street, Germantown.
 1902 McGowan, J. M., 406 South Broad Street.
 1894 McKee, James H., 1519 Poplar Street.
 1906 McLaughlin, J. J., 1813 South Broad Street.
 1905 Maier, E. G., 2242 North Broad Street.
 1905 Maier, F. Hurst, 2244 North Broad Street and 1900 Chestnut St.
 1902 Marshall, C. J., 2004 Pine Street.
 1899 Masland, H. C., 2134 North Nineteenth Street.
 1873 Meigs, Arthur V., 1322 Walnut Street.
 1906 Meyers, Milton K., 2134 North Eighteenth Street.
 1878 Mills, C. K., 1909 Chestnut Street.
 1906 Mitchell, Charlotte B., 1707 Pine Street.
 1884 Mitchell, J. K., 1730 Spruce Street.
 1907 Montgomery, C. M., 256 South Fifteenth Street.
 1898 Morris, Henry, 313 South Sixteenth Street.
 1885 Morrison, William H., 8021 Frankford Avenue, Holmesburg, Pa.
 1887 Morton, S. W., 1933 Chestnut Street.
 1899 Müller, G. P., 334 South Fifteenth Street.
 1906 Muschlitz, Chas. H., 3611 Spruce Street.
 1880 Musser, J. H., 1927 Chestnut Street.

 1879 Neff, J. S., Cynwyd, Montgomery Co., Pa.
 1900 Newlin, A., 253 South Thirteenth Street.

ELECTED

- 1898 Newton, R. D., 6137 Vine Street.
1902 Noble, C. P., 1509 Locust Street.
1900 Norris, Geo. W., 1530 Locust Street.
- 1894 O'Malley, Joseph, 2228 South Broad Street.
1900 O'Reilley, Charles A., 127 South Eighteenth Street.
- 1893 Packard, F. R., 1836 Pine Street.
1907 Pancoast, H. K., 4238 Pine Street.
1901 Patterson, F. D., 2103 Locust Street.
1900 Pearson, L., Veterinary Department, University of Pennsylvania.
1903 Pemberton, R., 1953 Locust Street.
1897 Pepper, William, 1811 Spruce Street.
1895 Perkins, F. M., 1428 Pine Street.
1897 Peter, L. C., 1700 Oxford Street.
1902 Pfahler, G. E., 1321 Spruce Street.
1885 Piersol, G. A., 4724 Chester Avenue.
1905 Piersol, Geo. M., 344 South Sixteenth Street.
1907 Pitfield, R. L., 5211 Wayne Avenue.
1890 Potts, C. S., 1733 Chestnut Street.
1908 Prime, Frederick, Jr., 344 South Sixteenth Street.
1901 Purves, G. M., 4204 Walnut Street.
- 1905 Radasch, H. E., 914 South Forty-seventh Street.
1904 Rahte, Walter E., 309 South Sixteenth Street.
1885 Randall, B. A., 1717 Locust Street.
1904 Reber, Wendell, 1212 Spruce Street.
1894 Reckefus, C. H., 506 North Sixth Street.
1906 Reichel, John, William Pepper Laboratory.
1907 Repplier, S. J., 328 South Sixteenth Street.
1894 Rhein, J. H. W., 1732 Pine Street.
1903 Rhein, R. D., 2016 Pine Street.
1907 Richardson, W. W., State Hospital for Insane, Norristown, Pa.
1894 Riesman, David, 1715 Spruce Street.
1891 Ring, G. O., 2012 Chestnut Street.
1904 Rivas, Damaso, corner Sixty-seventh and Vine Streets.
1876 Roberts, John B., 313 South Seventeenth Street.
1884 Robertson, W. E., 320 South Sixteenth Street.
1901 Robinson, E. T., 1326 Pine Street.
1904 Robinson, G. Canby, Pennsylvania Hospital.
1889 Robinson, William Duffield, 2012 Mount Vernon Street.
1908 Rodman, J. Stewart, 1904 Chestnut Street.
1901 Roe, W. J., 1210 Locust Street.

ELECTED

- 1898 Rosenberger, R. C., 2330 North Thirteenth Street.
1894 Ross, George, 1721 Spruce Street.
1900 Roussel, A. E., 2112 Pine Street.
1905 Royer, Franklin B., Municipal Hospital.

1895 Sailer, Joseph, 248 South Twenty-first Street.
1905 Salus, H. W., 1118 Pine Street.
1904 Sargent, A. Alonzo, 939 Spruce Street.
1890 Sartain, Paul J., 212 West Logan Square.
1895 Schamberg, J. F., 1922 Spruce Street.
1904 Schumann, E. A., 15 Pelham Road.
1882 de Schweinitz, G. E., 1705 Walnut Street.
1893 Scott, J. A., 1834 Pine Street.
1907 Sharpless, F. C., Rosemont, Pa.
1898 Sharpless, Wm. T., 100 South Church Street, West Chester, Pa.
1902 Shields, W. G., Jr., 412 West School House Lane, Germantown.
1905 Shoemaker, Harlan, 1618 Spruce Street.
1889 Shoemaker, Harvey, 2011 Chestnut Street.
1896 Shumway, E. A., 2007 Chestnut Street.
1899 Sinclair, J. F., 4103 Walnut Street.
1902 Sinkler, Francis W., 220 South Sixteenth Street.
1868 Sinkler, Wharton, 1606 Walnut Street.
1901 Siter, E. H., 2038 Locust Street.
1881 Skillern, P. G., 241 South Thirteenth Street.
1903 Small, J. H., 914 South Forty-eighth Street.
1903 Smith, A. J., Medical Department, University of Pennsylvania.
1906 Somers, Henry J., State Hospital for Insane, Norristown, Pa.
1902 Somers, Lewis S., 3554 North Broad Street.
1905 Speese, John, 328 South Sixteenth Street.
1893 Spellissey, Joseph M., 110 South Eighteenth Street.
1896 Spiller, William G., 4409 Pine Street.
1904 St. John, E. Q., 1833 Chestnut Street.
1890 Stahl, B. F., 1727 Pine Street.
1901 Stanton, W. B., 732 Pine Street.
1904 Stellwagon, Thomas C., Jr., 1121 Spruce Street.
1889 Stengel, Alfred, 1811 Spruce Street.
1889 Stevens, Arthur A., 314 South Sixteenth Street.
1896 Stewart, Alonzo H., 252 North Twelfth Street.
1899 Stout, G. C., 1611 Walnut Street.
1905 Stout, Philip S., 4625 Woodland Avenue.
1884 Strittmatter, I. P., 999 North Sixth Street.
1869 Stryker, Samuel S., 3833 Walnut Street.
1894 Swan, John M., 3713 Walnut Street.

ELECTED

- 1899 Talley, J. E., 1927 Chestnut Street.
 1897 Teller, Wm. H., 1713 Green Street.
 1906 Thomas, B. A., 1819 Chestnut Street.
 1894 Thomas, W. Hersey, 1421 North Seventeenth Street.
 1902 Thornton, E. Q., 1331 Pine Street.
 1902 Tracy, S. E., 1415 Walnut Street.
 1897 Tucker, Henry, 2000 Pine Street.
 1863 Tyson, James, 1506 Spruce Street.
 1890 Tyson, T. Mellor, 1506 Spruce Street.
 1900 Uhle, A. A., 1327 Jefferson Street.
 1904 Ullom, J. T., 24 Carpenter Street, Germantown.
 1894 Vandervoort, C. A., 3311 North Broad Street.
 1905 Van Kaathoven, J. J. A., 1715 Spruce Street.
 1903 Walker, J. K., 1632 Spruce Street.
 1891 Wallace, James, 1921 Chestnut Street.
 1899 Walsh, J., 732 Pine Street.
 1890 Warder, C. B., 1633 Spruce Street.
 1903 Weisenburg, T. H., 2030 Chestnut Street.
 1887 Westcott, T. S., 1833 Spruce Street.
 1908 Weston, Paul G., 1632 Green Street.
 1897 White, Courtland Y., 1808 Diamond Street.
 1873 White, J. William, 1810 South Rittenhouse Square.
 1901 Whiteway, Harold M., 1924 Chestnut Street.
 1893 Whiting, A. D., 1523 Spruce Street.
 1905 Wieder, Henry S., 2131 North Fifteenth Street.
 1868 Willard, De Forest, 1901 Chestnut Street.
 1898 Willson, R. N., 1708 Locust Street.
 1906 Wilson, J. D., Jefferson Medical College.
 1869 Wilson, James C., 1509 Walnut Street.
 1907 Wilson, Oscar H., 5128 Spruce Street.
 1891 Wilson, Samuel M., 1517 Arch Street.
 1901 Winsor, Henry, Manilla, P. I.
 1900 Wister, J. W., 5430 Germantown Avenue.
 1907 Wolf, Henry F., 1804 Green Street.
 1890 Wood, A. C., 128 South Seventeenth Street.
 1898 Wood, George B., 129 South Eighteenth Street.
 1906 Wood, Harold B., 5038 Pine Street.
 1865 Woods, D. Flavel, 1501 Spruce Street.
 1899 Worden, C. B., 322 South Sixteenth Street.
 1902 Zimlick, A. J., 702 East Cheltenham Avenue.
 1895 Zimmerman, Mason W., 1522 Locust Street.

NON-RESIDENT MEMBERS.

*Subscribers to Proceedings.

- Alburger, Henry R., Bloomington Indiana.
Ball, M. V., Warren, Pa.
Barnes, A. S., Jr., Missouri Trust Building, St. Louis, Mo.
*Biggs, M. H., Rutherfordton, N. C.
Carter, W. S., Galveston, Texas.
Coca, A. F., University of Pennsylvania.
*Dock, George, 602 East Huron Street, Ann Arbor, Mich.
Edwards, W. A., San Diego, Cal.
Egbert, Joseph P., Wayne, Delaware County, Pa.
Gaylord, H. R., Buffalo, N. Y.
Gerson, T. P., 1621 Ingraham Street, Los Angeles, Cal.
Hamaker, W. D., Meadville, Pa.
*Hamann, C. A., 404 Osborn Building, Cleveland, Ohio.
Harris, H. F., Atlanta, Georgia.
Harrison, W. H., Harrisburg, Pa.
Hatch, John L., 2010 Fifth Avenue, New York City.
Hickman, W. A., 612 Pacific Avenue, Atlantic City.
Holder, C. A., Colorado Springs, Col.
Howard, F. H., Williamstown, Massachusetts.
Hunt, J. R., 102 East Fifty-seventh Street, New York.
Jamar, John H., Elkton, Md.
Kiefer, Charles, United States Army.
Kinyoun, J. J., Glenolden, Pa.
Lincoln, W. R., Cleveland, Ohio.
*McConnell, G., 4175 Washington Avenue, St. Louis, Mo.
de Nancrède, C. B. G., Ann Arbor, Mich.
*Pearce, R. M., Bender Hygienic Laboratory, Albany, N. Y.
Pease, H. D., Buffalo, N. Y.

- Powell, W. M., 122 West Minor Street, West Chester, Pa.
*Rahter, C. A. 110 North Second Street, Harrisburg, Pa.
*Ravenel, M. P., University of Wisconsin, Madison, Wisc.
Reynolds, W., 27 South Indiana Avenue, Atlantic City.
Slifer, Henry F., North Wales, Pa.
Stadelman, Eugene, "Magistral," Maria Del Oro, Dgo., Mex.
Stubbs, R. P., Wilmington, Delaware.
Taylor, L. H., Wilkesbarre, Pa.
Toulmin, H., Haverford, Pa.
Wells, G. M., Wayne, Delaware County, Pa.
Wetherill, R. B., Lafayette, Ind.
Williams, H. L., Minneapolis, Minnesota.
Wilmarth, A. W., State Home for the Feeble-minded, Chippewa Falls,
Wisconsin.

CORRESPONDING MEMBERS.

ELECTED

- 1885 Dent, Clinton T., Assistant Surgeon and Lecturer on Practical Surgery at St. George's Hospital, Surgeon to Belgrave Hospital for Children, London, England.
- 1888 Fedeli, Gregorio, Rome, Italy.
- 1908 Flexner, Simon, Rockefeller Institute, New York City.
- 1890 Gibbs, Heneage, 585 John R. Street, Detroit, Michigan.
- 1908 Novy, H. G., Ann Arbor, Michigan.
- 1908 Osler, William, University of Oxford, England.
- 1886 Pye-Smith, P. H., Guy's Hospital, London, England.
- 1908 Taylor, A. E., University of California, San Francisco, Cal.
- 1898 Welch, William H., Professor of Pathology, Johns Hopkins University, Baltimore, Maryland.

Proceedings

of the

Pathological Society of Philadelphia.

NEW SERIES, VOL. XI.

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Observations on the Inoculability of Tumors and on the Endemic Occurrence of Cancer.

By LEO LOEB, M.D.

WHEN I was asked about twelve days ago to read a paper to-night I intended to make a small, quite unpretentious communication giving a few observations and experiments on tumor inoculation that I have made in the course of the last four months; but when, a few days later, I heard that the paper was to be discussed by some distinguished surgeons and pathologists, and that I was expected to speak somewhat longer than the usual time allotted, I saw that I must change my plans, it being too late to change the program, so I had to enlarge the scope of my remarks and give a brief outline of some of the work done in the last seven years in the experimental investigation of tumors, instead of reporting a few experiments I myself have carried out recently. After an interval of several years, I, only six months ago, obtained the opportunity of resuming my work. I will not try to go much into details, but rather to discuss some of the tendencies of cancer investigation at the present time.

I wish to say in the beginning that I have no startling announcement to make. The "Cause of Cancer" has not yet been discovered; but I do not, therefore, agree with those that, without much hesitation, condense their final conclusion into the short statement that we know nothing, nothing at all, about cancer. Such a statement is not very

encouraging for those who have given their energies to this work for some time; and what is more, I do not believe it is in accordance with the facts. We have learned something about cancer, and, much more than our actual achievements, we see the road clear for future work. We see problems on all sides, problems whose solution can be attempted successfully, and that present outlooks into new fields. That is the difference between the conditions now and as they existed eight or ten years ago. There is now a feeling of hopefulness among the workers in this field that was not present until quite recently. In Germany, France, England, and Russia new institutions for the study of cancer have been founded in the last few years. In America, which did not lag behind in this respect, Boston, Buffalo, and New York have now special laboratories for the investigation of cancer; and a steady increasing number of investigators are devoting their energies to this work. What has brought about the change? The increase in the number of cancer cases, which, as some investigators believe, exists? Hardly. Such an increase is far from being proved. I do not think I shall be far from the truth if I state that the generally awakened interest in cancer is due to the opening up of a new method of investigation—the experimental method. If I speak of the experimental method as a new one, I, perhaps, do not do full justice to the splendid work done by Hanau and Morau almost twenty years ago. The former transplanted cancer (a squamous epithelioma) in rats; and Morau, a carcinoma in white mice. This work proved that tumors can be transplanted into other animals. Hanau made very careful microscopic examinations of his transplanted tumors, and Morau made very interesting observations on the susceptibility of different strains of mice to the inoculation with carcinomatous material. These efforts, however, remained isolated, for pathologists were not yet prepared for this method of work. I may illustrate this:

About fourteen years ago I had the privilege of studying under Hanau, and the opportunity of speaking freely with him about problems in pathology; but he never so much as mentioned his experimental work on tumors, for he had long since passed to other problems. I did not even know at the time that he had done such excellent work in this field. The possibilities of the extension of the work were certainly not fully realized at that time. On the whole, the investigation of tumors was almost exclusively limited to microscopic studies.

Valuable and important as were the results given by these studies, there were noticeable the signs of exhaustion. The principal facts of the histogenesis and structural peculiarities of tumors arising from different types of tissues had been worked out as well as it could be done—in its main outlines as well as in many of its details.

The similarity between metastatic and original tumors was established. There is now very little doubt that the epithelial tumors usually arise from a direct downgrowth of the epithelial cells, and that this downgrowth is sometimes at first entirely unaccompanied with changes in the underlying tissue. In carcinoma proper, the changes are primarily in the epithelial cells. The origin of cancer may be unicentric and start from one point, and this seems to apply to the majority of cases; or it may be multicentric, originating simultaneously from several points. The growth of cancer takes place through the multiplication of its own cells, usually without infecting other cells of the body. These are the main results of anatomical and histological studies, and they are, on the whole, well established. There are still a few points about which some doubt is justified. Especially debatable is, for instance, the origin of certain so-called mixed tumors. It is possible that some of these, instead of being of embryonic origin, are due to the influence exerted by one growing tissue upon another, the latter starting to grow secondarily.

So far, purely morphological investigations could go; but they were not able to give us any further information. They could not give us any insight into the conditions of the growth of cancer, and could not bring us any nearer to the understanding of its cause. Attempts to prove the presence of a distinct and specific microorganism in cancer by morphological or cultural methods have, so far, failed. No convincing evidence of their existence could be obtained, although I am by no means ready to say that, among the various structures found in cancer and described by many authors, there may not have been protozoa. The attempt to fill with hypotheses the gaps where facts were missing has led to the constructing of many theories of the cause of cancer. Either these theories are enlargements upon one single fact, frequently in a purely speculative manner, or they are based upon erroneous observations as is the case with Ribbert's theory. There is no need to build up hypotheses prematurely, while we see before us a large field for work. First, let us find as many new facts

as possible and analyze these. There will be no difficulty in drawing our conclusions when we shall have sufficient data. These are what the more recent phase of the investigation of cancer promises to give us.

I refer to the experimental investigation of cancer in animals. That different animals may be afflicted with cancer has been long known, but our knowledge has been markedly increased within recent years. We are now able to make a certain number of generalizations, which are not without interest. In the first place, it has been established that not only parasitical pseudo tumors but that typical malignant tumors occur in cold-blooded animals, such as amphibia and fishes.

Secondly, some species of animals are much more frequently afflicted with cancer than others: cattle more frequently than sheep; dogs more frequently than either of these; mice and rats much more frequently than rabbits and guinea-pigs.

On looking over the character of different tumors that are found in different animals, I find that in certain species certain distinct types of tumor are prevalent. Eight years ago, I found that by far the most frequent variety of malignant tumors among cattle in America is the squamous-cell carcinoma of the inner canthus of the eye. In dogs the lymphosarcoma of the genital organs is very prevalent in America and Europe. In white rats, several sarcomata have been found by myself as well as by Velich, von Eisellberg, and Firket. Sarcoma seems, therefore, to be a relatively common tumor among white rats. This tumor appears, however, to be rare among white mice; but these animals are liable to another very common tumor—a subcutaneous carcinoma or adenocarcinoma, which, in many cases, at least, seems to take its origin from the mammary gland. This is by far the most frequent tumor in white mice. Among fishes, also, a certain type of tumor is characteristic for certain species, as the carcinoma of the thyroid in trout. We occasionally find, of course, other tumors in these animals, but those just named are the most common.

Fourth, a fact of great importance that has been observed in different animals is that tumors occur in them endemically. This means that tumors are very frequent among them in certain localities. In Wyoming I found, with Dr. Jobson, a ranch on which for ten years carcinoma of the eye has been endemic among the cattle, although the neighboring ranches are practically free from the disease. Among

caged animals the endemic occurrence of tumors has been found by Hanau, Morau, Borrel, Eberth and Spude, Michaelis, Gaylord and Clowes, and myself in the case of carcinoma, and by myself in the case of sarcoma.

Even among fishes the endemic occurrence of cancer has been observed during the last few years, having been very carefully described by L. Pick. It is well known that an endemic occurrence of cancer has also been found in man, and especially Behla, Lyon, and Sticker have investigated the conditions prevailing at such places very carefully. There is, however, one very important difference between the endemic occurrence of carcinoma in man and in animals, to which I called attention three years ago, namely, that while in man different varieties of tumors were observed in the affected area, in animals it was always precisely the same kind of tumor that was observed—namely, squamous carcinoma of the canthus of the eye in cattle, sarcoma of the thyroid in white rats, squamous-cell carcinoma of the vulva in Hanau's rats, adenocarcinoma of the subcutaneous tissue in mice, and carcinoma of the thyroid in fishes.

This is one of the reasons why the endemic occurrence of tumors in animals is so much more favorable for investigation than is the endemic occurrence of cancer in man. Furthermore, in animals we can vary the conditions of life at will much more readily than in man, in whom the number of variable factors is so much greater. In animals, which can be kept in small cages over long periods, the conditions can easily be arranged according to one's purpose. It is clear that an investigation into the cause of the endemic occurrence of cancer is of the utmost importance. Three sets of conditions have to be taken into consideration: (1) Hereditary influence, which could cause cancer to appear in certain families of animals; (2) infectious conditions—the transmission of an organism from one animal to another, either directly or by means of a host; and (3) environmental conditions—conditions of the food, water, etc.

My former observations did not permit me to decide definitely between hereditary and infectious causes. Some recent investigations of another case of the endemic occurrence of cancer in mice have suggested to me the existence of hereditary factors, but some known observations of others seem to point to an infectious cause. I refer especially to those of Haaland, from the Pasteur Institute, and to the

recent communications of Gaylord and Clowes. The latter observed 3 cases of sarcoma in rats confined in cages in which, several years previously, some of my sarcomatous rats had been kept for about five months. The question cannot, as yet, be regarded as absolutely decided. It is quite possible that a combination of factors is responsible for the endemic occurrence, but there is very little doubt that the problem can be decided in an exact way. It is necessary only to have the requisite means and enough time. There is no doubt, also, that such an investigation is of the utmost importance. It brings us in direct contact with the cause, or with one of the causes, of cancer. Here we see tumors new-formed in animals, without inoculation from another tumor. Ordinary tissue cells, such as the cells of the thyroid of a rat, are transformed, as in my case, into sarcoma cells, a long time after the animals previously affected with the tumor have been removed from the cage.

The same cannot be said of the second set of investigations to which I should like to call your attention. I mean the production of a tumor in an animal through inoculation with material from another tumor of the same species. In this case we do not convert previously normal cells into tumor cells, but we make tumor cells grow in other animals. The metastases of a tumor in the same animal can in this way be regarded as an autotransplantation of tumor cells. In a similar way as a cell or a multicellular organism can be produced only if there has been previously present another cell, so a tumor can under these conditions be formed only if there has been another tumor present at the start. Notwithstanding this fact, the experimental production of tumors through the inoculation of tumor material is of the greatest importance, inasmuch as it is likely to give us an insight into the conditions of the growth of tumors that could not be obtained in an exact manner in any other way, and, secondly, because it permits us to investigate the conditions under which an immunity against the growth of tumors can be produced in animals.

Now it might be argued that while transplantation into other animals can teach us the conditions under which cells grow after they have once been converted into tumor cells, and can show us the existence of immunity or predisposition to the growth of such tumor cells it can show us nothing about how normal cells become converted into tumor cells. I myself distinguished sharply between these two sets

of factors in my earliest investigations on tumor experimentation. Some experiences, however, suggest that there may exist a relation between the inoculability of a tumor and some of the conditions that favor the primary development of such a growth. In experiments on an adenoma of the mammary gland in a white rat, carried out in 1901, I found that the behavior of pieces of tumor when transplanted into the animal, affected with the primary tumor, was very different from their behavior when transplanted into other rats. The tumors grew or remained alive in the former; they died in the latter. Similar results were obtained in experiments that I carried out last winter with Dr. S. Leopold on a mammary tumor of a dog. In the course of repeated inoculations it was shown that pieces of tumor transplanted into the bearer of the primary carcinoma remained alive, but that they died when transplanted into other dogs. In a similar way, in the course of inoculations of tumors into white mice, one experiment showed distinctly that a mouse affected with a spontaneous tumor, similar to the one inoculated, evinced a predisposition to the growth of the inoculated tumor. The conditions that made this animal a favorable soil for the growth of the primary tumor favored the growth of cells that had already been converted into cancer cells. This is an important point that should be tested in future experiments; it leads to a further question, Can all tumors be equally well inoculated into other animals?

No, there exist differences. Certain tumors are much more likely to grow in other animals than are other tumors. Carcinomata, as well as sarcomata, have been successfully transplanted; other sarcomata or carcinomata could not be transplanted. On the whole, however, it seems probable that my prediction that sarcomata will be found more readily amenable to transplantation will be borne out by the facts; at least our experience so far tends to prove this. A certain percentage of tumors have been transplanted successfully. In the course of sarcoma of the thyroid in the rat, the readiness with which the tumors of the four rats afflicted with primary sarcoma of the thyroid could be transplanted, decreased, gradually. The sarcoma of the first rat could be transplanted most readily. The last sarcoma, with which I am now experimenting, gave the smaller number of successes. The others had an intermediate character. This is a remarkable fact which should lead to further investigation. In mice, a large

number of subcutaneous adenocarcinomata cannot be transplanted. Others of a similar structure can be readily transplanted and yield a large number of successful inoculations, while still others give only a very small number of successes. In dogs, the lymphosarcoma found in the genital organs is usually very readily transplanted, although other tumors of the dog show themselves refractory, in most cases, to successful inoculation. The three tumors mentioned are just the ones that have principally been used for inoculation, and I am in a position to-night to show you living animals affected with these three varieties of tumor. Ehrlich has also recently succeeded in a large percentage of cases in transplanting a chondroma found in a mouse.

If we now inquire into the causes of the difference existing in the inoculability of different tumors, we can mention a number of conditions that are of importance in that respect; but some work still remains to be performed. As I have already stated, I have investigated the differences in the inoculability of the primary cancerous animal and other animals carrying a similar primary tumor, on the one hand, and of other animals of the same species on the other hand. I found that males and females, young and old animals, can approximately be inoculated to an equal degree, but I also found that certain animals cannot be inoculated even when repeated attempts are made. Similar observations have been reported by Herzog, Jensen, and Ehrlich. Certain animals are refractory to inoculation with certain tumors. Michaelis, for instance, has made the interesting observation that certain strains of white mice may be more readily inoculated with carcinoma than others. There exists, therefore, a natural immunity in certain individuals against inoculation with tumor tissue.

I have recently given especial attention to the question whether pregnancy favors inoculation, but I have been unable to find any distinct influence. We see, therefore that in the first place, the predisposition or immunity of the animal is of importance, but, secondly, the character of the tumor itself has to be considered. Different tumors behave very differently in this respect, even when they have the same structure. I have mentioned the differences of this nature existing in the four sarcomata of the thyroid, which had an almost identical structure. Their inoculability was very different. The same holds good in the case of the carcinoma of mice. Many carcinomata in white mice are practically untransplantable. Three years ago I

transplanted a carcinoma in a Japanese mouse. Its structure did not materially differ from that of the carcinoma of the ordinary white mouse. I was able to transplant this tumor successfully in 100 per cent. of the cases. The lymphosarcoma in dogs can also be transplanted in almost 100 per cent. of the cases while other tumors of the dog cannot be transplanted at all, or only with difficulty. Perhaps we might be more successful if, for inoculation purposes, we could obtain the same variety of dog as the one in which the original tumor is found.

If we now analyze still farther the second set of factors, namely, those dependent upon the peculiarities of the tumor itself, we find that the energy of growth in the primary tumor is not the only factor that determines the inoculability of the tumor, although it is one of the factors to be considered. In the case of the carcinoma of the Japanese mouse, I found that in the original animal and in the first generation of transplanted animals it grew very slowly; nevertheless, it proved to be inoculable in 100 per cent. of the cases. I have recently observed that multiplicity of tumors does not necessarily favor inoculability. I was able to make this observation after having obtained simultaneously from one breeder three mice with multiple carcinoma. One of these animals had as many as nine tumors in different parts of the subcutaneous tissue or near by. We see, therefore, that the inoculability of tumors depends upon a large number of conditions which offer a fertile field for further investigation.

In this connection I may be permitted to draw attention to certain experiences, which are, perhaps, not without practical interest. I found in the case of my sarcoma in rats that the number of contact metastases in the animal operated upon depends upon the readiness with which the tumor can be inoculated into other animals. The first tumor, which could easily be transplanted, made the largest number of contact metastases. Approximately at the same rate as the inoculability decreased, the contact metastases decreased *pari passu*. The practically important point is this: We cannot determine beforehand from the structure of a tumor whether or not it is likely to make contact metastases. Two tumors may have a similar structure yet behave very differently in this regard.

Now let us consider the second phase of the experiment: A tumor has been found to be transplantable, and the next question is, How does it behave during transplantation into further generations? Does

the inoculability gradually decrease and does it end after a certain time? This does not need to be the case. My first sarcoma I transplanted into forty generations, and only accidental conditions prevented its further inoculation. Jensen's carcinoma has continually been undergoing propagation in several laboratories for the last five or six years, and there is no sign of diminishing inoculability as yet. I found in my first experiments that the transplantation of a very few cells, in mitotic division, present in the cystic fluid of the sarcoma, suffices to produce the largest tumors. This suggested to me, six years ago, the conclusion that ordinary somatic cells (ordinary connective-tissue cells or epithelial cells) have a much greater propagating power and the possibility of a much longer duration of life than had hitherto been supposed. It appeared to me possible that they might be immortal in the same sense as the germ cells are believed to be immortal. The experiences in tumor inoculation certainly suggest new lines of biological research that will be of great general interest. However, not all tumors can apparently be transplanted indefinitely, even if no accidental infection takes place. Morau noticed a gradual decrease in virulence in his tumors, although, even in this case, we cannot exclude with certainty the idea that accidental weakening influences, such as certain bacteria, had not been gradually inoculated simultaneously with the tumors.

In regard to the energy of tumor growth, some interesting conditions have been discovered to exist. As I pointed out two years ago, it is of frequent occurrence that the original tumor grows more slowly than the transplanted tumors. The maximum rate of growth may be reached in the second generation. This was especially marked in the course of my transplantations of the adenocarcinoma of the Japanese mouse, but I had noticed it in my early transplantations of a sarcoma. Other observers have made some similar observations.

As to the cause of this increase in the energy of tumor growth, I think it depends mainly upon the operative interference with the tumor through which it is possible to stimulate its growth. It is possible to obtain a similar stimulation of growth through cutting a piece of tumor or pulling a silk thread through the tissue. In this way I succeeded in causing tumors that had ceased to grow to start a fresh and vigorous growth. This experiment does not, however, succeed with all kinds of tumors. An adenocarcinoma of the breast of a dog,

with which I experimented last winter with Dr. S. Leopold, proved refractory in this respect. The effect of injury upon the tumor does not depend upon the production of a better blood supply, for on microscopic examination this was not found to exist in my specimens. It evidently depends upon a directly stimulating effect of the experimental conditions upon the energy of growth of the cells, and it explains the observations of surgeons that recurrent tumors may grow more rapidly than the original tumors.

It is also possible to diminish the virulence of tumor cells directly by subjecting them to certain physical or chemical conditions, *e. g.*, by heating the tumor cells up to 43° or 44° for one-half hour outside the body. Certain chemicals act in a similar way. This decrease in the energy of tumor growth is caused by a directly depressing effect upon the tumor cells, or upon the agency included among them that causes the growth. The important bearing of this fact upon some practical problems will soon be discussed. The knowledge that it is possible to decrease the energy of tumor growth experimentally, without killing the cells, came to me quite unexpectedly. The experiments of Sticker, Ehrlich, Apolant, and Michaelis have confirmed these results. If we increase the strength of the injurious influence but slightly, the tumor cells are killed. The depressing effect of certain external agencies was determined by me for sarcoma; and independently of me, at about the same time, by Jensen for carcinoma. It is interesting that there is scarcely any difference in this respect between sarcoma and carcinoma. There may, however, be a minor difference of about 2° or 3° in regard to the action of heat on different tumors.

Clowes and Baeslack have recently found that if the temperature to which the tumor is exposed outside the body before inoculation be still lower, and only slightly higher than the body temperature, the energy of tumor growth is increased. It is probable that this stimulus is of a similar kind to the one observed by me in threading a tumor or excising a part of it. Another method of increasing the energy of tumor growth has been used in recent years by Ehrlich. He uses, in such series of inoculations, the most actively growing tumor, and believes that in this way he has, by selection, obtained a race of tumors with increased virulence.

A question of great interest is whether inoculated tumors preserve their morphological characteristics during the course of their many

generations of life in other organisms. In general they do. Sarcomata, as well as carcinomata, occasionally, however, exhibit slight transitory variations as described by me in my first paper on transplantations of tumors. A sarcoma may assume a somewhat endotheliomatous structure, and the type of growth of an adenocarcinoma in mice may undergo slight changes; but, on the whole, the structure remains preserved. Only one very marked exception has been noted, namely, the appearance of a spindle-cell sarcoma in the course of the transplantation of a carcinoma, which I observed three years ago in a Japanese mouse, and which Ehrlich and Apolant noticed approximately at the same time. In my case the sarcoma originated as early as the first inoculated generation; in Ehrlich's it was found only in later generations. A fact of general interest is that in my transplantations the curve indicating the variations in the energy of growth in different generations applied equally to the sarcomatous and to the carcinomatous components. This suggests that the same factor underlies the growth of the sarcoma and that of the carcinoma. In both cases the carcinoma and the sarcoma could be mixed or entirely separated in the different animals. Do we have to deal with a transformation of carcinoma into sarcoma? Although there are places that might be interpreted as indicating such a transition, a careful study of the different tumors in very many sections makes it more likely that we have to deal with a conversion of connective-tissue cells into a sarcomatous growth. The epithelium transmits its stimulus to growth to the connective tissue of either the original animal or of the host. As I have mentioned above, these observations may throw some light upon the origin of such tumors as the sarcomcarcinoma of the thyroid. Instead of assuming that they are due to congenital malformation, we may, with as much justification, hold that primarily one tumor (a carcinoma) was present, and that only secondarily a stimulus emanating from the carcinoma caused the connective tissue to assume a sarcomatous growth.

We now turn to a question that is of theoretical, and promises to be of practical, importance, namely, that of immunity. In the foregoing I have already mentioned some facts that have a bearing upon this problem. We see that certain animals of a species are immune, while others are predisposed to the growth of an inoculated tumor. I have not infrequently observed, in the course of my tumor inoculations,

that certain tumors, after a preliminary growth, become stationary or may even retrogress. Mitoses may be present in these latent nodules for a long time, until mitotic divisions entirely cease to take place. Animals with merely stationary tumors may still constitute a favorable soil for tumor growth, as I found during my inoculations of sarcoma. Sticker, however, found, in the lymphosarcoma of dogs, that as soon as an animal has entirely recovered spontaneously from an inoculated tumor, it has become immune against any further tumor inoculation. Clowes and Baeslack observed the same fact in the case of carcinoma in white mice. They found, moreover, that the serum of such spontaneously recovered white mice has a certain, although only a very slight, immunizing effect against tumor inoculation upon other mice. Sticker likewise observed a certain curative effect of such serum upon other dogs affected with lymphosarcoma. These observations are probably to be explained in the following way: In a certain number of animals that seem to be predisposed to acquire an active immunity, the latter is acquired as the result of the temporary growth of a tumor. This immunity is an active one, and the serum acquires certain cytotoxic properties *in vivo*. This serum has, however, no agglutinating properties upon a tumor emulsion; certain antibodies are therefore lacking, as Sticker found.

But not all animals in which a tumor has been growing for some time have thereby become immune. Such animals may, as I very early observed in my experiments, be successfully inoculated with another tumor. Here I am able to show you a dog inoculated several months ago with lymphosarcoma, and reinoculated about four weeks ago. The second tumor is growing. Ehrlich has, however, recently found that when a mouse is inoculated with a very rapidly growing carcinoma, a second inoculation with a carcinoma will not be successful. In this case the animal has become immune through the first inoculation.

The next step was to find a method by which to immunize animals actively against the tumor growth. This applies as well to preventive immunity as to curative immunity. The principle that has to be applied for this purpose is the one announced by me more than three years ago in a paper before this Society, namely, the use of a virus of decreased virulence. I found especially effective for the production of such a vaccine a graded exposure to higher temperatures. Exactly

the same method has been successfully applied by Michaelis in the Berlin Institute für Krebsforschung within the last year. At first he used tumor tissue killed by chloroform for his experiments, but entirely without success. Neither was he able to obtain an active immunity by inoculating the tumors of gray mice into white mice, or vice versa. Ehrlich, however, obtained an active immunity, by using for inoculation, tumor material that was originally less virulent; for instance, hemorrhagic carcinomata in mice. Of theoretical interest is the fact that an active immunity against certain tumors of mice can be produced by injecting mice with different varieties of tissues of the mouse; for instance, embryonic tissue and sarcomatous tissue. Conversely the injection of carcinomatous tissue protects against sarcoma. Evidently we have here to deal with an active cytolytic property of the body acquired through the injection of cells of the same species. Practically, however, the inoculation of living-tumor tissue of ordinary virulence cannot be carried out in man, and von Dungern's experiments in the production of an active cytotoxic immunity, by injecting human milk into man, seems to have failed. Probably we shall again have to consider the use of a vaccine prepared by experimentally decreasing the virulence of tumor material. Very interesting results have been obtained by Sticker, who found in the lymphosarcoma of a dog that a single intravenous injection of a suspension of tumor material produces an active immunity in dogs without ever leading to the new formation of tumors. We see that we shall have to study carefully the conditions under which an active immunity can be produced in different animals before we can contemplate the use of such means in the case of human tumors, especially as it is quite probable that different varieties of tumors in animals and man behave somewhat differently in that respect.

As to a passive immunity, which means the use of the serum of actively immune animals for the cure of tumors, I can be brief. Since the first experiments of Richet and Héricourt, twelve years ago, many attempts have been made to cure tumors in man by the use of a serum. Especially von Leyden and Blumenthal are making and have been making for the last six years, systematic investigations in this direction, without, however, having produced very marked results. It is said that metastases do not develop under the influence of such serum. In mice Jensen has used the same principle, but his results have not

been of such a character that he could be convinced of the efficacy of the treatment. Other investigators have also tried it without having reported any definite results so far. Perhaps the future will give better results. It seems to me, however, that active immunity will be the method promising more success than the use of serum.

Time does not permit me to discuss certain recent investigations into the chemical character of malignant tumors. Definite statements in that regard can scarcely be made as yet, inasmuch as the cases examined have not been sufficiently numerous to justify definite conclusions. Certain differences seem, however, to exist between the chemical composition of tumors and that of the normal tissues from which they are derived. These differences are especially indicated by certain products found in tumor tissues undergoing autolysis.

There is, however, one other phase upon which I should like to touch briefly, and that is the difference between the behavior of normal tissue and that of tumor tissue during the process of growth. Normal tissue, when transplanted, if it grows at all, does so for only a limited period, and then the growth ceases. There seems to exist a certain cycle in the response of a normal tissue to a stimulus. I thought it possible that the action of the surrounding host tissue prevents the further expansive growth of the transplanted epithelium. Therefore, in the course of the last three years, I have carried out a large and varied number of experiments in which, at different stages of its growth, I liberated regenerating tissue from the influence of the surrounding connective tissue by re-transplanting it into other animals. This was repeated a number of times in succession. The result was identical in all the experiments; regenerating epithelium always retained its general properties. It remained regenerating tissue, and did not assume the expansive or infiltrating growth of tumor tissue. A summation of the stimuli, that might lead up to tumor growth, did not take place.

We may, therefore, conclude that under the influence of localized stimuli, of whatever character they may be, a malignant growth of cells cannot be initiated. Such cells, under the influence of localized stimuli, grow as long as the stimulus exists and then they return to their former state of equilibrium. Transplanted into another animal, in which the same stimulus would no longer be present, they behave like ordinary regenerating epithelium. We are forced to conclude,

from all the experiments that have so far been carried out on normal tissues, that a hereditary transmission of the changes produced by such stimuli upon certain cells cannot take place. Whether or not such an hereditary transmission does exist was one of the questions that I have tried to answer by the experiments in the successive transplantation of tissues. As we have seen, there is no indication that such an hereditary transmission can ever take place. Until this is proved we have to assume, in order to explain the growth of the malignant tumors, that can be inoculated into other animals, the constant presence of a certain stimulus in or around the tumor cells themselves—a stimulus that remains connected with the tumor cells into whatever animal these cells may be transplanted.

What I have just now said in regard to localized stimuli holds good of stimuli originating at points of the body farther distant. I may illustrate this with an observation that I have made in recent years: Toward the end of pregnancy the atresia of follicles in the ovaries of young guinea-pigs can assume a specific character, distinguished by a marked hypertrophy of certain cells. Such changes may be multiple. We have a right to believe, in this case, that a stimulus not unlikely of a chemical character is produced outside the ovaries, leading to this hypertrophic change in the follicles of the ovary. Transplanted into other animals, however, such changes would not progress in the follicles, because the stimulus that had initiated them would no longer be at work.

Localized stimuli, and those coming from more distant parts of the body, may lead to what might be called transitory tumors—tumor-like new formations that show a definite cycle in their development. They grow up to a certain point, so long as a certain stimulus is present, then they gradually retrograde. The corpus luteum may be regarded as the prototype of a transitory tumor. The small syncytiomata in the ovaries of very young guinea-pigs, which I found to be present in about 10 per cent. of the animals, also belong to that class. They reach a certain development, and then retrogressive changes take place. In a wider sense teratomata may likewise be placed in this class if they remain dormant, as well as other tumors that would cease to exist if transplanted into other animals in which a stimulus calling forth their proliferation would no longer be present. The course of such typical malignant tumors that seem to grow indefinitely

in other animals can, however, be explained only if we assume that the tumor cells carry with them the stimulus that causes their proliferation.

In conclusion, I wish to say that I believe that the tumor problem must at first be regarded as one of biology, and must be dissociated from any considerations of practical medicine. We have to analyze this problem as a part of the problem of growth in general, although any insight into the application of the knowledge we may gain through purely theoretical studies will certainly, in the end, benefit medicine.

April 11, 1907.

Etiology of Cholelithiasis; Bacteriological Study of 102 Calculi.

BY JOHN FUNKE, M.D.

(From the Laboratories of the Jefferson Medical College, Philadelphia.)

THE problem under consideration has engaged the attention of some of the foremost medical writers for a long time. Many of the factors bearing on the causation of gallstone formation have been fairly well settled, and it is merely connecting links that remain obscure; these to a certain extent seem of minor importance, but they have greater practical bearing than superficial thought would indicate. The question has been attacked from all sides, especially by the German and French investigators, and in order to understand clearly what has been done it is advisable to review some of the most important and recent studies.

Since the view expressed by Naunyn that bacteria play an important part in gallstone production most of the work on the question has been done with that factor in view. The first matter to be settled was whether or not normal bile contained bacteria. Here conflicting opinions are met, probably owing to differences in the bactericidal properties of human and animal bile, for, according to Mieczkowski, dried ox-gall inhibits the growth of bacteria to a greater extent than does human bile. Of the 55 normal animal gall-bladders examined, Miyake found but one infected. Naunyn and Netter both found the bile sterile in human beings at postmortem. Ehret and Stolz, on the other hand, conclude from their investigations that bile even

in the normal individual cannot be regarded as sterile. Perhaps the most significant work on this side of the question is that of Mieczkowski, who, at operation for lesions other than of the gall-bladder which was held to be in a normal state, found the bile sterile in the 15 cases examined. Undoubtedly more weight should be laid upon such investigations than upon animal experiments or the results of bacteriological examinations of bile obtained at autopsy; the latter give conflicting and unquestionably inaccurate findings, largely owing to the interval between the time of death and the necropsy. The most significant work which tends to support the findings of Mieczkowski and Miyake are the results of the postmortem investigation of Fraenkel and Krause, who made cultures at 128 autopsies and at two operations, and found the bile sterile 105 times. Before removing the livers from the cadavers these investigators ligated the cystic duct, then removed the gall-bladder and immersed it in a solution of bichloride of mercury for a few minutes, after which the gall-bladder was opened with sterile instruments and cultures made. Few investigators have been so successful; in the large majority of instances investigators find bacteria in the bile at postmortem. For instance, Letienne, at autopsy, found infection in 24 of the 42 cases examined. Gilbert and Girode, on the other hand, found the bile sterile at autopsy; the number of cases examined by these observers was small. In but 5 of the 12 cases I examined at autopsy was the bile sterile. The bile, like other body juices, may become contaminated with bacteria, and owing to the site of the gall-bladder perhaps more easily than the other fluids; so that the presence or absence of bacteria is of little importance if the walls of the gall-bladder are healthy and the duct leading from the organ is patulous, so that there is no impediment to the outflow of the bile.

Bacteria have been abundantly demonstrated in the bile during and after many diseases, especially typhoid fever. Much work in this connection has been done by Chiari, Talma, Fuetterer, Sailer, Pratt and Forster, and Kayser. Occasionally the *Bacillus typhosus* was found in the gall-bladder even when there were no lesions in the ileum, bacteriemia alone being present.

I wished to know whether the bacteriological examination of gall-stone nuclei might not bring to light some factor or factors concerned in the production of cholelithiasis. Before undertaking the work

upon which this paper is based I was aware of the fact that bacteria had been found in biliary calculi, but I was desirous of determining the result of the bacteriological examination of a large number of stones.

The calculi were first washed in a solution of bichloride of mercury, 1 to 500, and then in sterile water, after which they were crushed by means of sterile instruments and the pulverized nuclei inoculated in tubes of bouillon. Spreads were made of each pulverized nucleus, stained, and examined at once for bacteria. In order to ascertain whether or not the bile salts and the bile acids in the calculi would inhibit the growth of organisms inhabiting the stones, I inoculated tubes containing pulverized biliary concretions with the colon bacillus and found that even if dilutions were made through three and four tubes the bacteria always grew.

Of the 102 calculi examined, but 31 gave rise to growths in the bouillon; 71 inoculations remained sterile. Only now and then were bacteria found in spreads, and occasionally, perhaps in three calculi, were bacteria found in the spreads when no growth from the same calculus developed in the bouillon. From the infected tubes the colon bacillus was isolated 11 times in pure culture. The *Bacillus typhosus* was found once in pure culture and never associated with other organisms. The following organisms were isolated once: occasionally they were in pure culture, occasionally associated: *Bacillus limbatus*, *Bacillus gasoformans*, *Bacterium oxygenes*, *Bacillus Friedlander*, *Bacillus lactis aërogenes*, *Micrococcus tenacatis*, and *Bacillus subtilis*. The last-named organism was found in pure culture in eight of ten stones taken from the same case. The *Sarcina luteum* was found in four calculi, always associated with one or more organisms; the *Micrococcus cereus albus* was isolated twice. One calculus yielded a bacillus which could not be identified; it resembled the *Bacillus brookeri* very closely.

Soft concretions yield bacteria invariably. Although the bacteria from spreads of the nuclei which gave negative cultures stained fairly well, it is still a question whether or not the bacteria were viable at the time the inoculations were made; the retrogressive changes in the organisms may not have been sufficiently advanced to interfere with the staining reaction. Similar observations have been made by Gilbert and Dominici, and by Fournier, who found organisms

in three spreads made from three different calculi, the inoculations from which remained sterile. The last-named author succeeded in developing growths from 47 of the 70 calculi examined. Of the 17 concretions examined by Pratt, 4 contained the *Bacillus typhosus*. Gilbert and Dominici observed that recently formed calculi contain bacteria almost constantly, while the older ones are more frequently sterile. But 8 of the 27 calculi examined by Gilbert and Fournier were infected; one of their 27 concretions was obtained at autopsy and contained the colon bacillus. The five stones examined by Dufourt were sterile. Blumenthal found the *Bacillus typhosus* and the paratyphoid bacillus in the bile and in the concretions of a case of cholelithiasis.

The finding of bacteria in recently formed concretions in a very much greater percentage than in older ones would seem to indicate their presence in the bile at the time the calculi were formed. In attempting to solve the problem, whether or not calculi are bacteria-free, the concretions utilized for the experiments should be obtained at operation and examined at once, for I believe drying causes either death or diminution in the propagating capacity of the organisms, and it is perhaps responsible for the difference in the percentages of the infected calculi obtained by different observers. This opinion is based upon my observation that when I divided the concretions obtained at operation into two groups, examining one group immediately and allowing the other to dry for some weeks, a large percentage of the first group would always yield bacteria. While the almost constant presence of bacteria in recently formed calculi appears to indicate their presence in the bile at the time of the calculus formation, the experiments of Gilbert and Fournier and of Chauffard furnish sufficient ground to refute such a conclusion. These investigators have succeeded in demonstrating secondary penetration of the calculi by placing a concretion in a sterile bouillon tube, then subjecting it to a temperature of 75° C. an hour, daily, for three days, at the end of which time they inoculated the tube with the colon bacillus. The tube was then incubated at 37° C. for five days, when the surface of the concretion in the tube was sterilized, crushed, and reinoculated into a sterile tube of bouillon where the colon bacillus developed. They also succeeded in demonstrating that the organisms travel in the other direction. This phenomenon was illustrated by first

sterilizing the surface of the calculus thoroughly and then placing it in a sterile tube of bouillon, where it was allowed to remain for several days at a temperature of 37° C. At the end of the allotted time the bouillon was infected. These observations may explain the frequency with which soft calculi are inhabited by organisms. The finding of sterile bile and infected calculi at the same operation can scarcely be held to controvert secondary penetration, for the bile may have become bacteria free after having infected the concretions. In one case I found the bile obtained at operation sterile, while eight of the ten calculi obtained at the same time were infected with the *Bacillus subtilis* in pure culture. Although secondary penetration cannot be doubted in the light of the experiments quoted, yet there are few authors who hold that the presence of bacteria in the concretions must in all instances be regarded as such.

With the view of determining the cause of gallstone production much work has been done, and it would be superfluous to review in detail the literature, as this has recently been done so thoroughly by Herter and Lartigau. It must become clear to anyone who studies the subject that, perhaps, the first essential factor for the formation of calculi, as laid down by Naunyn, is impediment to the outflow of the bile. Such a state of affairs first permits infection, and secondly prevents the actively contracting gall-bladder from expelling the organisms. Miyake found that if he injected a pure culture of the colon bacillus into a gall-bladder from which the bile flowed freely, stones did not form. Ehret and Stolz, after inserting foreign bodies, sterile and infected, into the gall-bladder, noted that in order to infect the bile there must be a slowing of the outflow. Beer, in cases of extra-hepatic and intrahepatic lithiasis, either found impediment to the outflow present at the time of observation or he found evidence of its having been present some time previous. He ligated the common duct, infected the gall-bladder, and then found that cholelithiasis developed. Hartmann tells us that stagnation of the bile enhances the development of bacteria. Mignot found that if sterile, foreign bodies were introduced into the cystic duct, so as to prevent the outflow, the bile remained sterile and no concretions formed; he also found that if infected foreign bodies were introduced into the gall-bladder, precipitates formed. He concludes that retardation of the flow of bile is a necessary factor in the production of calculi. This

fact had already been recognized by Friedrich Hoffmann toward the latter part of the first half of the eighteenth century. Perhaps it is unnecessary to have complete obstruction of the bile duct, or even partial occlusion, for muscular weakness of the gall-bladder may produce the same effect as obstruction. In this manner, or by a similar method, the retardation of bile is brought about by predisposing factors associated with cholelithiasis. Thus, we can trace direct connection to such conditions as changes in the intra-abdominal pressure following repeated pregnancies, gastrectasis, upward displacement of the right kidney, tumor in surrounding organs and in the biliary ducts, constipation, sedentary habits, obesity, and cholelithiasis. With regard to the diet, Hoppe-Seyler tells us that the production of intestinal catarrh is perhaps the only influence indiscretion in diet might have as a predisposing factor in gallstone production.

Should the bile become stagnant or even sluggish in its flow, infection usually follows. How readily this may be accomplished becomes evident when we consider that the portal circulation frequently carries bacteria, and that an enormous amount of blood is taken to the liver, from which organ the bacteria may be excreted through the bile. Many investigators have ligated the cystic or the common duct, then in the course of a very little time demonstrated bacteria in the bile above the occlusion or in the gall-bladder. The most extensive work in this direction has been done by Ehret and Stolz, Miyake, Mignot, and Mieczkowski. Bond, by means of fistulae in the gall-bladder, was able to demonstrate indigo granules in this organ after the administration of the drug by mouth. From these findings, he concludes that if the flow of bile becomes sluggish, even non-motile bacteria may gain access to the gall-bladder through the biliary channels. Doerr found soon after intravenous injection that the bacteria appear in the gall-bladder and in the stomach, but if injected subcutaneously or intraperitoneally they did not reach the gall-bladder.

The experimental production of biliary calculi in animals has been successfully accomplished by Mignot, Gilbert and Fournier, Miyake, Lartigau, and also by Ehret and Stolz. These experimenters have succeeded only when certain laws were obeyed, as, for instance, they are unanimous in the assertion that there must be either occlusion of the common duct or an impediment to the outflow of the bile from

the gall-bladder. Wolynzew alone states that he failed to produce biliary calculi even when the laws laid down by the investigators just quoted were observed. In experimental work the impediment to the outflow of the bile has been furnished by the introduction of foreign bodies into the gall-bladder. It is held that even though the occlusion of the ducts is not accomplished, the foreign bodies in the gall-bladder will cause sufficient stagnant bile to ensure adequate infection to set up an inflammatory process, the latter being, of course, the essential factor in the production of cholelithiasis. Mignot noted that in order to succeed in the production of gallstones attenuated cultures by bacteria must be employed to cause the infection, since virulent organisms nearly always cause death of the animals. Thus it appears that a catarrhal inflammation is the desired effect. It has been noted by other writers and experimenters that cholelithiasis is comparatively infrequent with suppurative conditions of the gall-bladder, although Beer claims to have seen intrahepatic lithiasis consecutive to suppurative cholangitis. The consensus of opinion is that the cholelithiasis is responsible for the suppurative lesions. Catarrhal conditions are perhaps necessary for two reasons: (1) In order to secure sufficient mucins and pseudomucins to agglutinate the salts formed during the inflammation; (2) in this condition the epithelial cells undergo a set of changes degenerative in nature, during which cholesterin is elaborated, whereas, if the infection be severe, death of the cells is rapid, and distinct necrotic rather than degenerative changes result. It is by no means definitely proved that the cholesterin is a derivative of epithelium, but if we seek the cause of this salt in other parts of the body it is found to occur in areas of degeneration, especially in the nervous system. It is also a frequent content of cysts. Then, too, if one takes the trouble to examine biliary calculi, most of them will be found composed largely of cholesterin. Some of the most ardent advocates of the view that cholesterin is formed from the epithelial cells of the gall-bladder maintain that the bile before reaching this organ contains very little cholesterin, and that it is added at this point. This view is supported by the work of Jacobsen, who found that the bile taken from a fistula in the duct contained 0.56 part of cholesterin per mille, and by the more recent work of Hammersten, who found that the bile from the gall-bladder contained more cholesterin than the bile taken from the ducts through fistulæ.

The bile from the gall-bladder contained from 0.87 to 0.99 part per mille while that from the biliary fistula contained from 0.05 to 0.15. Both investigators were working with human bile. The results of these workers seem to corroborate Naunyn's view that the cholesterin found in gallstones is largely produced in the gall-bladder. Whether or not this is a result of changes in the bile produced by infection and whether the soaps holding the cholesterin in solution are so altered that precipitation occurs are still questions for debate. Letienne and Girode say that if a solution of biliary salts be saturated with cholesterin and then inoculated with the colon bacillus, precipitation of the cholesterin occurs. I have repeatedly placed bile obtained at autopsy in a test tube and introduced the colon bacillus; in nearly all instances precipitation occurred, but never cholesterin. In the majority of instances the only formed elements were needles of fatty acids. Herter tells us that marked alkaline reaction of the bile favors the precipitation of bilirubin, yet the microorganisms most frequently responsible for infection of the gall-bladder are acid producers. The weight of evidence seems to point to the fact that the salts contained within biliary concretions are largely a product of the diseased mucous membrane. Bramson, however, holds that the amount of calcium salts ingested influences the amount of this salt in the bile and plays a part in the production of cholelithiasis.

With regard to the agglutinating property of bile in causing clumping of the *Bacillus typhosus*, as put forth by Cushing, there appears to be very little material to offer in support. I am strongly inclined to the belief that such a phenomenon plays little or no part in gall-stone production. Taking for granted that the bile does agglutinate the *Bacillus typhosus*, it would still require a catarrhal inflammation to elaborate the necessary salts to form the calculi, and if such a condition of the gall-bladder existed the debris resulting from the destroyed or degenerated cells would furnish material more suitable to act as a nucleus of the concretions than the clumps of bacteria. If the *Bacillus typhosus* were the only organism causing the condition, the hypothesis might carry with it more weight; but it has been experimentally proved that other bacteria can accomplish the same result, and perhaps do so more frequently.

Notwithstanding the fact that infection of and impediment to the outflow of bile are necessary factors in the causation of gallstones, it

is also true that infection of the gall-bladder occurs without cholelithiasis. I have seen at autopsy occlusion of the cystic and common ducts and infection of the bile in the gall-bladder, but no stones. There are perhaps other factors still obscure, which play an important part in the production of biliary concretions, and I believe that these factors must be worked out upon a combination of clinical and postmortem data, and that experimental investigation cannot clear up the obscurity.

Accessory Pancreas, with Report of Two Cases.

By A. G. ELLIS, M.D.

(From the Laboratories of the Jefferson Medical College Hospital.)

THIS anomaly of the pancreas is not of common occurrence, but in recent years, since more careful histological studies are made of autopsy material, instances are being quite frequently added.

Warthin,¹ in 1904, collated 47 cases and added 2. I have found references to 14 others reported before or since Warthin's paper was published. Gandy and Griffon,² in 1901, described a specimen found in the first part of the duodenum. Reitmann,³ in 1903, reported 2 cases, one in the wall of the ileum 10 cm. from the ileocecal valve, the other in the duodenojejunal flexure. In 1904 Turner⁴ described a nodule of pancreatic tissue situated in the jejunum 30 cm. from its origin. In the same year Drs. Miller and Alburger each presented a case before this Society, and in the discussion which followed personal cases were cited by Drs. Robinson, Longcope, and Stengel.⁵

Bize,⁶ also in 1904, added 2 cases, each specimen being at the extremity of a diverticulum of the ileum, 25 and 60 cm. respectively from the ileocecal valve. He cites a case reported in 1899, by Brunner, the nodule being in a diverticulum 37 cm. above the valve. Bize also cites 4 cases, all in diverticula, reported in 1891 by Heller and Schmauser, but as his references are not clear, and those cases are mentioned neither by Glinski nor Thorel, I have not included them. In 1905 Lewis⁷ found an accessory pancreas in the jejunum 85 cm. below the pylorus. Hedinger,⁸ in 1906, described a specimen situated at the tip of a Meckel's diverticulum. To these cases I am enabled to add 2, making a total of 65.

Naturally the larger number of these ectopic organs are found along the course of the gastro-intestinal tract. Since in 4 instances 2 specimens each were found, the 65 cases include 69 nodules of pancreatic tissue, located as follows: stomach, 17 (one in a diverticulum); duodenum, 14; jejunum, 21 (one in a diverticulum); ileum, 11 (eight in diverticula); intestine, 1; Meckel's diverticulum, 1; umbilical fistula, 1; mesenteric fat, 1; omentum, 1; hilus of spleen, 1.

The unusual situation of one of the specimens I present and the probable beginning malignancy of the other is considered justification for putting on record these 2 cases. Both were found within a period of three weeks.

CASE I.—This specimen was obtained at autopsy upon a male infant, a twin, 37 cm. long, who died two hours after premature birth due to placenta previa. The findings were essentially those of a normal child. In the hilus of the spleen and firmly attached to that organ was a lobulated, yellowish body 5 mm. in diameter, supposedly a lymph node.

Microscopic sections passing through both spleen and nodule show the latter to be an accessory pancreas. Some of the acinar cells are in a fair state of preservation, others are degenerated. A few small interlobular ducts are present. At a few points are what appear to be indistinct centro-acinar cells, and there are also several circumscribed masses of small cells resembling islands of Langerhans. Fibrous tissue is not abundant, but several bands divide the gland into distinct lobules. The nodule is attached to the spleen by a relatively broad band of loose connective tissue containing numerous large blood-vessels.

CASE II.—The second specimen was found 5 cm. from the pylorus in the posterior wall of the stomach of a colored man aged eighty-six. It was in the shape of a solid, grayish, slightly elevated nodule, 1.5 cm. in length and 0.6 cm. in width. Microscopically the submucosa contains two small areas of pancreatic tissue quite widely separated and forming but a small part of the nodule. In both parts are ducts, with one exception small in size and possessing no demonstrable communication with the gastric mucosa. Many of the acinar cells are detached from the wall; most are granular, some are partially disintegrated. A few acini contain what are regarded as centro-acinar cells. Islands of Langerhans are not present. Connective tissue is scanty

in the large area, but in the small are several broad bands dividing it into lobules.

Separating the two masses of pancreatic tissue, and at points extending beyond them, is a much larger area made up chiefly of tubules lined by tall cylindrical epithelium. Thick bands of connective tissue divide this area into several fairly distinct lobules. In each, the centrally situated tubules, appearing mainly in longitudinal section, are very greatly dilated to form irregular cyst-like spaces with numerous finger-like extensions. At several points the lining cells are supported by no demonstrable basement membrane, and in a few instances appear quite clearly to be penetrating the surrounding connective tissue, though the latter nowhere is extensively invaded. Toward the periphery of these lobules the tubular epithelium shows degenerative changes and at outlying points, but continuous with them are smaller, regular tubules or acini lined by cuboidal cells in advanced stages of degeneration. A few tubules lined by cylindrical cells are at isolated, widely separated points in the muscular coat of the stomach.

From the general structure of this nodule it appears reasonable to infer that the central area is an adenomatous overgrowth of the ducts, with extensive atrophy and degeneration of the pancreatic tissue. One of the specimens described by Bize contained a similar adenoma. Of the greatest importance in my case is the relation of the epithelium of this growth to the surrounding tissue, this as described justifying the diagnosis of potential, if not actual, carcinoma. Warthin considers the possibility of malignancy as the most important single point regarding the pathology of these islands of ectopic tissue. To this question my second case adds affirmative evidence not hitherto adduced, and hence, furnishes a distinct contribution to the pathology of accessory pancreas.

Of further interest in this case was the presence in the greater curvature of the stomach near the cardiac end of a second nodule 1 cm. in diameter, which on section proved to be a fibromyoma occupying all of the muscular coat and a part of the submucosa. Muscle fibers are most conspicuous in the peripheral portions, the centre being made up entirely of dense fibrous tissue. This nodule contains no sign of pancreatic tissue.

These accessory organs appear of little or no physiological value. Their capability when the normally situated pancreas is by disease

rendered partially or entirely functionless must, however, be considered. From a pathological standpoint their possibilities are more numerous, though mainly remote. They undoubtedly favor diverticulum formation, Bize probably being correct in his assumption that all diverticula containing pancreatic tissue are produced by traction of that structure, and hence are in no wise true Meckel's diverticula. Intestinal obstruction may be caused by these just as it is by the ordinary Meckel's diverticulum, and was so caused in Brunner's case and the second one of Bize. Intussusception, fistula, fat necrosis, and portal of entry of infection are other possible untoward effects, but in the great majority of cases the ectopic tissue has appeared to be entirely harmless.

The simplest and withal the most reasonable explanation of the origin of these misplaced fragments is that during the development of the pancreas cell masses are separated from the organ and carried to variable distances by further growth of the tissues. This readily explains the accessory masses in the wall of the stomach and intestines, they having been carried upward or downward by longitudinal extension of the growing canal. For those in the omentum, mesentery, or region of the spleen this exact method cannot be active. Endres believes that detached fragments may be carried in the tissues to a distance by arterial growth, as along the course of the gastro-epiploic or pancreaticoduodenal vessels, in this way even lodging in the stomach wall. This would hardly account for the specimen attached to the spleen, and we must conclude that, as in the case of the adrenal and other organs, there are various ways in which ectopic fragments are formed and placed.

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Significance of Tubercle Bacilli in the Feces.

By RANDLE C. ROSENBERGER, M.D.

(From the Clinical Laboratory of the Philadelphia Hospital.)

THE detection of tubercle bacilli in solid or well-formed stools of several patients, whose histories were vague and in whom the clinical diagnosis was far from clear, led the writer to take up this subject for research.

The idea was to actually see how prevalent the occurrence of acid-fast bacilli in the feces was by studying the feces of others than those suffering from known tuberculous infection. With this end in view, during the past two years 612 cases were collected from the wards of the Philadelphia Hospital. Together with this number there were stools of 60 cases of diagnosticated tuberculous infection, making in all 672. The stools were obtained from patients with croupous pneumonia, typhoid fever, erysipelas, diarrhea, surgical and nervous cases, and from individuals who were apparently healthy; in fact, from patients in all wards of the institution, no matter what the clinical diagnosis. Of these stools, 137 were solid, 297 were semisolid, and 178 were fluid. The tubercle bacillus was found in 120 cases, or 19.6 per cent.; in solid stools 28 times, in semisolid stools 40 times, and

in fluid stools 52 times. In the 60 cases of diagnosticated tuberculosis the organism was demonstrable in all, no matter what the consistency of the feces.

It has been observed by some that acid-fast bacilli have been found in the feces of those suffering from enteric fever, and that it is quite common for them to be found in all specimens of feces, even in health; that this latter assertion is absolutely erroneous will be shown by the results of the studies here recorded. The presence of the tubercle bacillus in the feces may be a process of excretion, so a few experimental studies regarding the excretion of bacteria in general may be quoted.

Newmann (*Berl. klin. Woch.*, 1890, p. 229) and Karlinski (*Prager med. Woch.*, 1890, p. 231) observed the *Bacillus typhosus* in the urine in 35 out of 112 cases examined. Wyssokowitsch (*Zeits. f. Hyg.*, 1886, vol. i, p. 3, quoted by Sherrington), in 22 experiments, employed; 14 bacterial species, only twice were the species introduced into the blood, and yet they were found in the contents of the intestine. On each occasion there were macroscopic hemorrhages in the serosa and mucosa. He concludes that the passage of bacteria into the excreta occurs only when the blood containing them escapes through some breach of continuity in the excretory membrane due to inflammatory or mechanical injury. Dobroklouski (quoted by Sherrington, *Jour. Path. and Bact.*, 1893, p. 258), in Cornil's laboratory, found that the bacillus of avian tuberculosis, when administered with food, infected guinea-pigs by penetrating the healthy intestinal mucosa.

As corroborative evidence, numerous investigators have performed experiments by feeding, by subcutaneous and intravenous inoculations, and proved that the organisms reach and pierce the mucous membrane without any evident lesion being present.

Emmerich and Buchner (*Arch. of Hyg.*, vol. ii, p. 357), after injecting the *B. neapolitanus* into the blood, found that the organism escaped in large numbers through the intestinal wall. In one experiment no pathological change in the intestinal wall was observable, but in almost all there was blood in the contents of the intestine and hemorrhages in the mucosa. Sherrington (*Jour. Path. and Bact.*, 1893, p. 276), in concluding his article on the escape of bacteria with

the secretions, says the evidence is against believing that when the transit of bacteria across the secreting membrane occurs the membrane is still normal in condition, although at the same time it may not be ruptured or pervious to red blood cells.

The fact that the escape of the bacteria tends to occur not immediately upon the introduction of them wholesale into the circulation, but in the late stages of the communicated disease, suggests that the healthy secreting membranes are not pervious to bacteria, and that only after soluble poisons produced by the infection have had time to act upon them do the membranes become pervious to the germs.

Babes (*Comp. Rend.*, Paris, 1888) concludes from experimental work upon glanders that the bacillus can penetrate uninjured mucous membranes. At this point may be mentioned the well-known experiments of Ravenel, who, shortly after having fed animals tubercle bacilli, killed them and found the bacilli in the thoracic duct, though no lesions or abrasions of the mucous membrane of the gut were evident.

As another instance of the penetration of mucous membranes by pathogenic organisms, typhoid bacilluria may be mentioned. This condition was studied at some length by Koujojeff (*Central. f. Bact. u. Parasit.*, 1889, vol. vi, p. 672), by Charrin and Ruffer, Ruffer, Schweiger, Blachstein, Corrado, Pernice and Scagliosi, Cornil, and others quoted by Sherrington (*loc. cit.*). They have proved by experiments upon animals with various bacteria that it is quite common for bacteria to be found in the bile or urine after intravenous or subcutaneous inoculation, or even through feeding.

I inoculated rabbits and guinea-pigs subcutaneously with a homogenized culture of attenuated tubercle bacilli. Upon the fourth day I detected tubercle bacilli in their feces. After seven weeks the animals were killed. The guinea-pig showed neither a local nor visceral lesion, not even intestinal lesions, while the rabbit, though no visceral lesions were found, had a caseous mass at the site of inoculation and in the right inguinal region. A second series of guinea-pigs and rabbits were inoculated in the same manner and with the same organism. I again found tubercle bacilli in their feces at the end of the fourth day. These animals were killed at the end of the

sixth week, and at autopsy no intestinal, visceral, or glandular lesions were present in the guinea-pig, but the rabbit showed a large, apparently caseous mass in the right inguinal region, in which I was unable to find tubercle bacilli.

Examination of a number of specimens of feces from guinea-pigs and rabbits in apparent health failed to reveal any acid-fast bacilli.

In well-defined instances of pulmonary tuberculosis it is the rule to find tubercle bacilli in the feces. If a case comes under the observation of the clinician which is not at all clear, presenting a clinical picture resembling malaria, enteric fever, or acute miliary tuberculosis, the finding of tubercle bacilli in their feces will determine the diagnosis.

In those suffering from chronic diarrhea, with no other appreciable symptoms, pulmonary or otherwise, the tubercle bacillus is at fault in most cases. These cases have frequently come to autopsy, some showing intestinal ulceration and others showing no ulceration.

Instances of ascites, the exact nature of which was unknown, were diagnosticated positively as tuberculous by the finding of the tubercle bacillus in the feces. These cases were further proved by surgical procedures, *i. e.*, abdominal operation. As a well-marked intestinal tuberculous ulcer is appreciable from the appearance of the serous coat of the gut, in one case thus operated upon no ulcers were present.

That the tubercle bacilli are in the feces, irrespective of a pulmonary or an intestinal lesion, is proved by the fact that I found them in cases of general glandular involvement, meningitis, hip-joint disease, and in Pott's disease of the spine, the latter condition being in a boy, aged five years. In acute miliary tuberculosis, diagnosticated or not diagnosticated, clinically, the bacillus was present in the feces in all cases. The result of these studies suggests the intestinal mode of infection in tuberculosis in general. It is my intention merely to quote a few observations supporting the theory of the intestinal infection in tuberculosis, as these studies strengthen that theory. This theory of infection is gaining ground daily, and the experiments of Schroeder and Cotton (*Bull. No. 86, Bureau of Animal Industry of Dept. of Agriculture*), of Ravenel and of Vallee, who practically confirmed Ravenel's feeding experiments by working upon calves;

of von Behring, of Calmette and Guérin's work upon goats, demonstrate fully that the intestine is by far the most common path of infection, and that aërial infection is uncommon.

In a number of autopsies, in which the mesenteric and other glands were studied bacteriologically, it was found that over 40 per cent. showing no tuberculous lesions in any part of the body were tuberculously infective. It was also found that in all cases of active tuberculosis, and in almost all cases of inactive tuberculosis, the mesenteric glands were tuberculously infective (Rosenberger, "A Study of the Mesenteric Glands in Their Relations to Tuberculosis," *Amer. Jour. Med. Sci.*, July, 1905). Supporting von Behring's theory that the most frequent method is through the intestinal wall, Guthrie found over 22 per cent. of infections through the intestinal tract, Heller nearly 40 per cent. of primary intestinal affections, and Still over 23 per cent.

To explain the presence of the tubercle bacillus in the feces of man is not easy.

The writer believes that the bacillus enters the human economy through ingestion, water (?), either in infancy or maturity. At first the number of bacilli is not large, and they find their way to the blood and lymph stream. During their transit some are discharged through the feces and others through the urine.

The circulation of these organisms through the lymph and blood continues indefinitely, and the patient actually suffers from a toxemia which may be so severe as to set up chronic diarrhea, or cause vague symptoms characteristic of no one disease.

As the organisms multiply in the body, and as the toxemia and irritation progress, a point of least resistance is somewhere established, the tubercle bacillus lodges and sets up the disease with its distinct pathological features. It is interesting to note that in cases of healing tuberculosis, or actually arrested or healed cases, the tubercle bacillus is rarely if ever found in the feces. This is also true of the occurrence of tubercle bacilli in the sputum. In some of these cases recorded, the sputum has been examined at least six and as many as twelve times negatively and subsequent examination of the feces was also negative.

Passler (*Munch. med. Woch.*, October 23, 1906), in considering

the diagnosis of pyretic conditions, as septicemia and typhoid, mentions the probability of acute intestinal tuberculosis as the cause of the malady, irrespective of tuberculosis of any other organ. During the evolution of intestinal tuberculosis, he suggests that the infection by pyogenic organisms will perform the same role as they do in ulcerative pulmonary tuberculosis, and asserts that when we are dealing with a marked pyretic condition, which has lasted for several weeks without any particular and definite signs to indicate typhoid fever or septicemia, we must always think of an acute intestinal tuberculosis. He concludes by saying that as an intestinal infection may occur without any manifestations clinically, the feces should be examined for the presence of tubercle bacilli. (He cites only two cases.)

Wood (*Chemical and Microscopic Diagnosis*, 1905) states that tubercle bacilli are found in the feces of persons suffering from tuberculosis (pulmonary), because in the majority of cases the bacilli are swallowed, together with small masses of sputum.

Sahli (*Diagnostic Methods*, 1905) mentions that in intestinal tuberculosis tubercle bacilli are found in the feces, and are therefore of diagnostic importance. The stools may, however, contain these bacilli even though there is no intestinal tuberculosis (if the patients swallow their sputum). Searching the stools has even been recommended for the diagnosis of lung tuberculosis in cases of irresponsible persons who swallow the sputum. Previous treatment with dilute potassium hydroxide or digestive enzymes is often successful and may be serviceable in the examination of mucopurulent particles of the movement which have been isolated from the mass of feces. We do not know whether under certain conditions decomposition will destroy tubercle bacilli in the intestine. At any rate, we cannot always demonstrate tubercle bacilli in the stools, even when there is undoubted intestinal tuberculosis. Perhaps this is on account of the dilution of the content of tubercle bacilli by the abundant particles of food. Tubercle bacilli are most readily found in the purulent or bloody pieces of diarrheal stools. As tubercle bacilli in the feces may be due to swallowed sputum, we can diagnosticate intestinal tuberculosis if bacilli are found in the feces only when at the same time attacks of diarrhea occur with pus and blood in the

stool. The tubercle bacillus must be carefully distinguished from the smegma bacillus, which is said to occur at the anal orifice and might have become mixed with the feces.

Lichtheim (*Fortschritte Med.*, January, 1883) says that the presence of tubercle bacilli in the stools is the exception rather than the rule in persons suffering from pulmonary tuberculosis. He further asks the question whether the presence of these organisms in the feces means intestinal ulceration or merely that the patient has swallowed them with his sputum. In control observations he showed this not to be the case, as he claimed it was only exceptional to find the bacilli, as they were very difficult to find, and then only a very few were present.

In January, 1897, Shaw (*Jour. Amer. Med. Assoc.*, March 20, 1897, p. 554) mentioned the finding of tubercle bacilli in the feces of a patient exhibiting no tuberculous lesion of the intestinal tract. The lungs, however, showed isolated tubercles and areas of bronchopneumonia.

Emerson (*Clinical Diagnosis*, 1906, p. 390), in remarking upon the occurrence of tubercle bacilli in the feces, says that it must always be borne in mind that the organisms may be swallowed, and this especially so in children in whom the diagnosis of pulmonary tuberculosis has been made; "but this is rather a remote possibility in the case of a careful adult."

Boston (*Clinical Diagnosis*, 1904, p. 380) recommends collecting a "small portion of the purulent or mucoid material from the feces, smear it thinly on a slide," and then stain for tubercle bacilli, as in the sputum. "Tubercle bacilli when found in the feces point conclusively to the existence of tuberculous ulceration of the intestines."

Simon (*Clin. Diag.*, 5th ed., 1904, p. 328) claims that when tubercle bacilli are present in the feces it indicates intestinal ulceration, providing they are observed upon repeated examinations and there are clinical symptoms pointing to the bowels as the seat of the disease; otherwise, they may be referable to swallowed sputum.

TECHNIQUE.—If the specimen to be examined was a fluid or semi-solid one, a small quantity from any part of the stool was taken and spread on a slide, dried, and stained. When the feces were solid,

a small amount of sterile distilled water was put upon the slide and a small mass of fecal matter mixed thoroughly, spread, dried, and stained. Not one of the specimens was centrifugalized.

In staining the preparation, carbol fuchsin was applied for fifteen minutes in the cold, the excess drained off, and Pappenheim's solution poured on the preparation. This was allowed to act for two or three minutes, washed with water, and if the specimen was of a uniform blue color it was dried and examined in cedar oil. If the preparation was not uniformly blue, Pappenheim's solution was applied and reapplied until the smear was blue. By observing this technique carefully no mistake can happen regarding the diagnosis of the tubercle bacillus, as this organism and spores of other bacilli are the only bodies retaining the carbol fuchsin stain. All other bacteria and cellular elements are stained blue. Great care must be taken lest some artefact be mistaken for the tubercle bacillus, such as a minute scratch in the glass, a small crystal, or the periphery of a cell. The organisms, as a rule, are comparatively few in cases not plainly diagnosed as tuberculous, but in well-marked cases of pulmonary or intestinal tuberculosis they are comparatively abundant. The finding of the tubercle bacillus in a smear is not always easy of accomplishment; it has frequently taken the writer at least an hour, and sometimes as long as two hours, to find three or four bacilli.

Direct searching through solid stools is less promising than in fluid stools. Nevertheless, tubercle bacilli may quite frequently be demonstrated in solid movements if, as Hamburger (quoted by Sahli) recommends, we mix a piece of feces the size of a pea with a few centimeters of water, then centrifuge gently to remove the coarser pieces, dilute the supernatant cloudy fluid with a double volume of alcohol, centrifuge once more, and then after drying examine the remaining precipitate, which will consist almost exclusively of bacteria. (Personally, I have never found this procedure necessary.)

Park (*Pathogenic Microorganisms*, 1905, p. 312) recommends searching in the feces for any purulent or mucous particles, and if none are found the larger masses are removed, the rest diluted and centrifugalized, and stained by the ordinary methods.

Page (quoted by Emerson) mixes a small mass of feces in 1.5 c.c.

distilled water, adds 54 c.c. of a mixture of equal parts of alcohol and ether, centrifugalizes ten minutes, makes a smear of the sediment, fixes it to the slide with albumin, and stains as usual.

If the assertion that tubercle bacilli found in the feces result from swallowing sputum, or the presence of intestinal tuberculosis is true, the examination of the feces is useless, as no further knowledge is gained.

It is a well-known fact to students of tuberculosis that a persistent diarrhea is present for a very long time, and yet the autopsy shows no ulcerations in any part of the intestinal canal. Therefore, it is by no means pathognomonic that, if we find tubercle bacilli in the feces of those suffering from tuberculous enteritis, ulcerative lesions are present. But suppose that acid-fast bacilli are present in the feces of a person not suffering from any appreciable lesion of tuberculosis. What then is the significance of such a finding?

To the writer it has been proved from the studies made of this number of cases, both from a clinical and pathological standpoint, that if an acid-fast bacillus is present in the feces of any individual, and this organism resembles morphologically and tinctorially the tubercle bacillus, tuberculosis of some part of the body exists.

I do not mean pulmonary tuberculosis, but tuberculosis of the intestines, liver, lymph nodes, peritoneum, or any viscus. A *resume* of some of the cases that came to autopsy, with general remarks of those who did not succumb to the disease, is of great interest and very instructive.

In almost two-thirds of the cases I was fortunate in being able to follow up the clinical findings with those at autopsy. A small percentage of cases were removed from the institution in a precarious condition, and although a number of cases died, no autopsy was permitted.

I wish to extend my thanks to the various resident pathologists at the Philadelphia Hospital for their valuable coöperation in the work.

CONCLUSIONS.—1. No other acid-fast bacillus was found in the feces but the tubercle bacillus.

2. The presence of the tubercle bacillus in the feces means that active tuberculosis exists somewhere in the economy.

3. In acute miliary tuberculosis the bacillus is always present in the feces.

4. In all cases of chronic diarrhea and in cases of general glandular involvement the feces should be examined for tubercle bacilli.

5. The finding of tubercle bacilli in the feces does not mean intestinal ulceration in all cases.

6. In arrested or healed pulmonary tuberculosis no tubercle bacilli are found in the sputum or feces.

7. The feces should be studied for tubercle bacilli as a part of the routine examination, especially in suggestive cases and where no expectoration can be obtained.

Resume of cases in which tubercle bacilli were found in the feces irrespective of the clinical diagnosis, with findings at autopsy, and with general remarks on those that did not come to the autopsy table:

1. Clinical diagnosis, cirrhosis of liver with ascites. There was no expectoration. Three days before death delirium set in, suggesting meningitis. At autopsy, miliary tuberculosis of lungs and tuberculosis of the peritoneum; no intestinal ulcerations.

2. Clinical diagnosis, chronic pleurisy. Eleven examinations of sputum were negative. At autopsy pleura was one-eighth of an inch in thickness over right lung, and the same lung showed miliary tuberculosis; no intestinal ulcerations.

3. Clinical diagnosis, acute miliary tuberculosis. Sputum examined on nine occasions with negative results. At autopsy miliary tuberculosis of lungs and spleen; no intestinal ulcerations.

4. Clinical diagnosis, acute miliary tuberculosis. At least six examinations of the sputum were negative for the tubercle bacillus. At autopsy there was found acute miliary tuberculosis of all the viscera; no intestinal ulcerations.

5. Clinical diagnosis, pleurisy and tuberculosis of hip. Sputum on a number of occasions was negative for the tubercle bacillus. At autopsy there was acute miliary tuberculosis of all viscera; no intestinal ulcerations. (Three weeks after finding the tubercle bacillus in the feces it was found in the sputum.)

6. Clinical diagnosis, typhoid fever. No expectoration, and there were four negative Widal reactions. At autopsy, six small irregular atypical ulcers, long axis transverse to bowel; one of these ulcers

had perforated the gut. Studied bacteriologically, these ulcers showed few tubercle bacilli.

7. Clinical diagnosis, tuberculosis of the hip. No autopsy.

8. Clinical diagnosis, acute miliary tuberculosis. Acute miliary tuberculosis of all viscera found at autopsy but no intestinal ulcerations.

9. Clinical diagnosis, croupous pneumonia and pleurisy. No tubercle bacilli found in the sputum. Crisis occurred, but the patient is still running an irregular temperature.

10. Clinical diagnosis, chronic diarrhea. No cough, no expectoration. No autopsy.

11. Clinical diagnosis, chronic diarrhea. After finding tubercle bacilli in the feces, slight impairment of resonance was noted in the right apical region.

12. Clinical diagnosis, chronic diarrhea and cirrhosis of the liver. No expectoration. Tuberculous ulcers were found in the gut at autopsy.

13. Clinical diagnosis, repeated attacks of pleurisy. No expectoration. No autopsy.

14. Clinical diagnosis, pleurisy with effusion. Sputum negative on four occasions for the tubercle bacillus.

15. Clinical diagnosis, chronic diarrhea. No expectoration. Typical tuberculous ulcers of the gut were found at autopsy.

16. Clinical diagnosis, erysipelas and diarrhea. No expectoration. Slight pulmonary lesions were found; no intestinal ulcers.

17. Clinical diagnosis, septicemia? pelvic abscess? typhoid? Acute miliary tuberculosis of all viscera; no intestinal ulcers.

18. Clinical diagnosis, malignant endocarditis. No expectoration. Acute miliary tuberculosis of all viscera; no intestinal ulcers.

19. Clinical diagnosis, chronic diarrhea. At autopsy, intestinal ulcers were found.

20. Clinical diagnosis, alcoholism. No expectoration. Recent and old lesions were present in the lungs; no intestinal ulcers.

21. Clinical diagnosis, tuberculous peritonitis. At autopsy, tuberculous peritonitis and intestinal ulcers were found.

22. Clinical diagnosis, pleurisy. Tuberculosis of both pleuræ,

of mesenteric glands and spleen; no pulmonary or intestinal lesions found.

23. Clinical diagnosis, chronic diarrhea. Tuberculous ulcers present.

24. No clinical diagnosis made. At autopsy general miliary tuberculosis was found, but no intestinal lesions.

25. Clinical diagnosis, enteritis. This case was a child, aged two years, treated for seven months for gastro-enteritis; there was no cough, no expectoration; the cervical glands were enlarged.

26. Clinical diagnosis, tuberculous peritonitis. Patient had cavities in both lungs, yet no sputum could be collected.

27. Clinical diagnosis, Pott's disease. This case was a child, aged five years; no pulmonary lesions demonstrable. No autopsy.

28. Clinical diagnosis, typhoid fever and pneumonia. No tubercle bacilli were demonstrable in the sputum on several different occasions, and during illness two negative Widal tests resulted, and blood showed a leukocytosis of 17,000. At autopsy, acute miliary tuberculosis of both lungs, liver, and spleen, with few intestinal ulcers.

29. Clinical diagnosis not made. At autopsy, general miliary tuberculosis; no intestinal ulcers.

30. Clinical diagnosis, tuberculous peritonitis. This case was operated upon and the diagnosis confirmed.

31. Clinical diagnosis, probable carcinoma of the stomach. Pernicious vomiting, chronic diarrhea, marked cachexia, and emaciation were among the cardinal symptoms. No autopsy.

32. Clinical diagnosis, typhoid fever. Child, aged six years, running a persistent irregular temperature, no pulmonary symptoms, and three Widal tests were negative.

33. Clinical diagnosis, secondary anemia. Besides the tubercle bacillus being found in the feces, the ova of the tricocephalus dispar and ascaris lumbricoides were present. The malarial parasite was also seen in the blood. (As this was an immigration case, he was immediately deported.)

34. Clinical diagnosis, probable tuberculosis of the liver. Bacilli were found on two occasions in the feces. No autopsy.

35. Clinical diagnosis not made. Sputum examination negative

on three occasions. Caseous tuberculosis of the bronchial and mediastinal glands; miliary tuberculosis of the kidney, liver, spleen, and intestines was found at autopsy.

36. Clinical diagnosis, chronic pleurisy. No tubercle bacilli were found, in the sputum. At autopsy adhesive pleurisy on both sides was found, together with a few miliary tubercles in the lung. There was no intestinal ulceration.

37. Clinical diagnosis, erysipelas. No tubercle bacilli were demonstrable in the first three examinations of the sputum, though a fourth examination was positive. (Three days after tubercle bacilli were found in the sputum pulmonary hemorrhage took place and the bacillus was found at this time.)

38. Clinical diagnosis, interrupted recovery from typhoid fever. No autopsy.

39. Clinical diagnosis, typhoid fever. Acute miliary tuberculosis of general character was found at autopsy; no intestinal ulcerations.

40. Clinical diagnosis, chronic diarrhea. Tuberculosis of the intestines and tuberculosis of the mesenteric glands was present at autopsy.

41. Clinical diagnosis, acute miliary tuberculosis. No tubercle bacilli were found in the sputum. At autopsy acute miliary tuberculosis of all the viscera was found, but no intestinal ulcers.

42. Clinical diagnosis, chronic diarrhea and jaundice. X-rays showed gallstones. Tuberculosis of the retroperitoneal glands was observed at autopsy, but no gallstone.

43. Clinical diagnosis, pneumonia. No crisis, and running an irregular temperature. No tubercle bacilli could be found in the sputum. Healed tuberculosis of the lungs, with tuberculous ulcers in the gut, was found at autopsy.

44. Clinical diagnosis, typhoid fever. No expectoration. At autopsy acute miliary tuberculosis of a general character was found.

45. Clinical diagnosis, carcinoma of the liver. At autopsy cancer of the lung and liver was found, together with tuberculosis of the mesenteric glands.

46. Clinical diagnosis, locomotor ataxia, with empyema. One liter of pus was found in the pleural cavity at autopsy. There was

one positive and two negative examinations of the sputum for tubercle bacilli.

47. Clinical diagnosis, general glandular enlargement. Spreads from an inguinal gland removed during life showed tubercle bacilli, and spreads from a mesenteric gland removed at autopsy also contained tubercle bacilli. At autopsy there was no pulmonary tuberculosis, but a purulent peritonitis, with general enlargement of all lymphatic structures. No intestinal ulcers.

48. Clinical diagnosis, typhoid fever. At autopsy, tuberculous ulcers were observed; no pulmonary lesions.

49. Clinical diagnosis, tuberculous peritonitis. Operation disclosed fibrinopurulent peritonitis. Numerous miliary tubercles on visceral and parietal layers of the peritoneum were observed, but no intestinal ulcerations could be made out.

50. Clinical diagnosis, malaria. Patient has had chills, fever, and sweats occurring almost daily for ten days. Examination of the blood on several occasions failed to demonstrate the malarial parasite.

51. Clinical diagnosis, meningitis. Child, aged eighteen months. No pulmonary symptoms. Tubercle bacilli were demonstrable in the spinal fluid about the same time they were found in the feces.

52. Clinical diagnosis, cervical lymphadenitis. Upon the second examination, and after a very careful and prolonged search, a few tubercle bacilli were found in the feces.

53. Clinical diagnosis, cutaneous tuberculosis (lupus). Although tubercle bacilli were found in the feces, no pulmonary lesions could be made out.

54. Clinical diagnosis, diarrhea alternating with constipation. No autopsy.

55. Clinical diagnosis, gunshot wound of the chest penetrating the lung. On five occasions the sputum was examined for tubercle bacilli, but with negative results. At autopsy a large cavity was found which was surrounded by gangrenous tissue, and on the margin, upon histological examination, tubercles were found which showed few tubercle bacilli.

56. Clinical diagnosis, tumor of testicle, probably tuberculous. The organ was removed, and histologically presented a typical picture

of tuberculosis. No pulmonary lesions were evident. It might also be mentioned that tubercle bacilli were found in sections of the organ and also in spreads before fixation.

57. This case was one in which a large ulcerating mass was present in each groin, involving the inguinal glands. The condition had persisted for two years. Tubercle bacilli were found in the feces, though the condition had been diagnosticated as sarcomatous, specific, and tuberculous. Sections of the masses studied histologically showed typical tubercles and giant cells and tubercle bacilli.

Besides these cases just cited there were nine cases of chronic diarrhea, certain of which, upon autopsy, showed tuberculous ulcers and others did not. In a few of these latter cases slight pulmonary involvement could be seen, while in the greater number no pulmonary lesions could be made out.

DISCUSSION.

DR. W. T. LONGCOPE wished to know whether the bile of tuberculous patients had been examined for tubercle bacilli. This question was suggested to him by the fact that typhoid bacilli were so frequently found in bile.

DR. DAVID RIESMAN asked whether the feces of patients with lupus had been examined for tubercle bacilli.

DR. W. W. HAWKE referred to a case in the Insane Department of the Philadelphia Hospital. A man who had been gaining in weight for the past two years had developed what was thought to be a rectal irritation from seat worms. Physical examination revealed a friction in the upper right quadrant of the abdomen which was thought to be produced by the colon slipping over the surface of the liver. Two specimens of the feces were examined and tubercle bacilli found in both. There were no definite outspoken signs of tuberculosis, though a suspicion of an attenuated form of tuberculosis was being entertained.

DR. JOSEPH MCFARLAND asked whether other tests than the morphology and staining characteristics had been used to prove that the organisms found were tubercle bacilli. He thought it incon-

ceivable that tuberculous lesions so obscure that definite signs were not present should discharge bacilli into the intestinal tract in such numbers as to be found as reported.

DR. H. R. M. LANDIS, referring to similar examinations on the feces of tuberculous patients at the Phipps Institute, stated that it was his opinion that practically all patients with pulmonary tuberculosis swallow some tubercle bacilli. These could be detected in the feces, showing that tuberculous ulcerations in the intestine were not necessary to have the bacilli in the stools.

DR. ROSENBERGER, in answer to questions and in closing, stated that seventeen specimens of bile from tuberculous patients had been examined, with negative results. He had only been able to secure one case of lupus without pulmonary lesions, and this one had shown tubercle bacilli in the feces. No other test than the morphology and tinctorial characteristics of the organism had been applied to the bacilli found. Several attempts had been made with animal inoculation, but all had failed, owing to the death of the animal from septicemia. The organism most apt to be confused with the tubercle bacillus would be the smegma bacillus, and this could be differentiated by the method used. Pappenheim's solution after staining with carbol-fuchsin will decolorize this organism in twenty minutes.

Squamous-celled Carcinomata of the Esophagus.

By W. TAYLOR CUMMINS, M.D.

(From the Pathological Laboratory of the University of Pennsylvania.)

WITH regard to the type of cell, carcinomata of the esophagus are divided into two classes, the squamous-celled and the columnar-celled. The former are much more frequently found, for, in fact, cases of the latter type are rarely encountered. For the most part the tumor is primary in this organ, but cases are reported in which it is the seat of metastatic deposits from the pharynx, thyroid, and cardia of the stomach. The esophagus appears to be invaded but rarely by cancerous growth, for out of a series of 722 cancers¹ in all parts of the body

only 6 were found in the esophagus. It seems to enjoy a certain degree of immunity from neoplastic invasion in contrast with the organs lower in the alimentary tract. Zenker and von Ziemssen² have collected reports of 5079 autopsies, of which 0.36 per cent. showed esophageal cancer, and of these 0.25 per cent. were primary.

The organ may be divided very conveniently into three segments, viz., an upper or cervical, a middle or thoracic, and a lower or diaphragmatic portion. Bland-Sutton³ believes that the location of the neoplasm may determine whether it is of the squamous-celled or columnar-celled type, the former electing the upper two-thirds and the latter the lower third of the tube. This statement must not be made dogmatically, owing to the fact that statistics reveal many instances in which the squamous-celled tumor primarily involved the lower third of the organ. As to the point of greatest frequency of involvement there seem to be widespread differences of opinion. It is conceded that the points of narrowing of the tube are the usual seats of the new-growth. These are found at the levels of the cricoid cartilage, the bifurcation of the trachea and of the diaphragm. Possibly localized trauma at these apparently stenotic areas may incite tumor formation. Upon making a *resume* of the statistics at hand there is revealed the fact that the new-growths, including both types, are somewhat more frequently found in the lower third of the organ. Kraus⁴ collected 901 cases, and of these 397 were found in the lower third, 302 in the middle, 158 in the upper third, and 45 involved more than one part of the organ.

The esophageal tumor may be small and definitely circumscribed, or, on the other hand, it may be quite large, with imperfect demarcation. In some instances multiple foci have been observed. There is usually some stenosis of the tube, but in a few reported cases this condition was absent. Ulceration and cicatrization are likely to develop and in many cases the lumen of the gullet is almost obliterated. In those cases in which the carcinoma involved the diaphragmatic segment, Bland-Sutton has explained the forcible ejection of food after swallowing by the fact that the tube assumes a spindle shape on account of the stenotic condition and there occurs an hypertrophy of the muscular walls immediately above, thus favoring a forcible regurgitation of the esophageal contents.

The squamous-celled cancer is found much more frequently in men than in women. Bland-Sutton has observed it four times more frequently, while Mackenzie, Zenker, and von Ziemssen have found it three times more frequently in men. The distribution in the sexes appears comparable to that of neoplasms of the stomach. Age seems to be a factor in its production. The prolific period is between forty and sixty years, while cases are recorded as early as the thirtieth year, and as late as the eighty-fourth year. A few exceptional cases are on record in which the disease appeared in the nineteenth and twenty-first years. Curiously, the female sex seems to be attacked earlier in life than the male.

It has been said that carcinomata of the esophagus do not often metastasize. This has been explained by the fact that the disease is often rapidly fatal and metastases have not had the opportunity to develop. Certain it is that the patient, in many instances, is not long under observation subsequent to the development of localized symptoms, and death may take place from inanition, exhaustion, or septic pneumonia. Reports of metastatic growths are noted rather infrequently. The posterior mediastinal glands appear to be affected more frequently than any of the other structures, and their position renders easy access of tumor tissue from the thoracic segment of the gullet. Of 55 cases⁵ of esophageal carcinomata recorded at St. George's Hospital, London, these glands showed metastatic deposits in 24 cases. Evidences of metastases were observed in the liver in 10 cases, in the lungs in 6 cases, in the kidneys in 5 cases, in the bones in 4 cases, and in the adrenals and spleen in 2 cases each. Widespread dissemination of the cancerous elements is distinctly rare. In a few cases reported in literature the tumor had existed for years without producing secondary foci, and they were generally of the flat-celled type.

Among those who have reported upon cancer of the esophagus with metastases are Burnet,⁶ Butlin,⁷ Wright,⁸ Scott,⁹ and Eskridge.¹⁰ Cancer statistics have been collected by Gillies¹¹ and Moak.¹²

PERSONAL OBSERVATIONS. These have been confined to a review of the autopsy records on file at the Pathological Laboratory of the University of Pennsylvania from 1874 to 1907. The number of records examined was 1993, and of these 10 showed squamous-celled carcinomata of the esophagus, distributed as follows: 1 in 1891, 1 in 1893,

4 in 1901, 1 in 1904, 2 in 1906, and 1 in 1907. In regard to the prevailing sex, the males outnumber the females, 8 to 2. This conforms with the assertions made by the other investigators that these neoplasms are much more frequent in men than in women. The ages ranged from thirty-four to seventy-four years. The youngest cases aged thirty-four and thirty-eight years were females, while the youngest male was fifty. It appears that the disease may develop earlier in life in the female than in the male. In but 6 of the 10 cases was the race noted, and all of them were white. Nothing conclusive appears in literature with regard to the comparative frequency of the condition in the races. As to the points of predilection in the esophagus, as already stated, there appear to be differences of opinion. In 7 cases only was the site of the primary tumor indicated, and 4 of these involved the lower third of the tube. Some of the statistics are based upon all cancers of the esophagus, while personal observations were made upon the squamous-celled type alone. The results are comparable from the fact that the indifferent type, the columnar-celled, is so infrequently encountered. All of the tumors of this series appeared to be primary in the esophagus, and involvement of not more than one segment had occurred. As to the general character of the tumors, 4 showed considerable ulceration, 3 were fungoid, and 1 was characterized by dense cicatrization of the esophageal walls. In 2 cases the appearance of the tumor was not noted on the records.

The number of cases presenting metastases outnumbered those without 6 to 4. This surely does not show a comparative rarity of metastases in such tumors. For the most part, the notes upon the gross anatomy of the organs were disregarded, and the diagnosis was determined by the histological findings. Metastatic growths occurred in the organs with the following frequency: Stomach, four times; liver, three times; pancreas, three times; lungs, twice; posterior mediastinal glands, twice; bronchial glands, twice; hepatic glands, twice; kidneys, once; pancreaticosplenic and lumbar glands, each once. The most widespread metastases occurred in Case II, in which foci were found in the liver, lungs, stomach, pancreas, hepatic, pancreaticosplenic, and bronchial glands. Histologically the primary and secondary growths were divided into two groups dependent upon the presence or absence of "epithelial pearls." In view of the fact that

the keratinous structure of the squamous epithelium of the esophagus is poorly developed, the presumption might be that tumors involving such a tissue would usually show an absence of "pearls." Such was not the case in this series, for 5 showed the presence and 3 the absence of "pearls." In 2 cases this point was not noted in the records.

It seems plausible that the facts pertaining to the development of the tumors of the spinal and basal cellular types of carcinomata of the skin might readily apply to these tumors, *i. e.*, the tumors in which the superficial strata of epithelium are directly concerned show "epithelial pearls," while those in which the epithelium of the deeper or basal strata has proliferated show no "pearls." In the secondary as well as in the primary growths the nests of tumor cells were much smaller in those cases showing "epithelial pearls" than in those in which they were absent. In several of the metastatic deposits in the latter group the squamous character of the cells was made out with some difficulty. Those cases which showed "pearls" in the esophageal tumor showed the same in the secondary tumors. However, there appeared a tendency toward a diminution in size, and this was well shown in the tumor of the kidney (Case VII), in which the "pearls" could with difficulty be recognized. It was thought by the author that a comparison of the frequency of metastases in those primary tumors with and without "pearls" might reveal less frequent metastases in those with "epithelial pearls." Examination of the records reveals that of the 4 cases without metastases 3 showed "pearls." So limited a number of cases makes it problematical, but at least suggestive.

Several of the tumor sections presented rather unusual features. In one, a section of lung, there was shown embolism of the smaller radicles of the pulmonary arteries by masses of squamous epithelium. The deposits were confined to these locations, and but few of them were seen. In a pancreas there was infiltration of a small lobule by squamous epithelium, with the Island of Langerhans vaguely evident. In a kidney the cortex showed a large, irregularly rounded mass composed of small nests of squamous epithelium with very small, deeply stained "pearls."

METASTASIS. Under this heading a brief description of the lymphatic system of the esophagus and neighboring structures is indispensable. The lymphatics of the esophagus fall into two groups—viz.,

those in the submucosa and those in the muscular coats. The cervical portion of the organ drains into the superior deep cervical and recurrent nodes. The lymphatics draining the middle or thoracic segment pass to the posterior mediastinal glands, while those of the lower or diaphragmatic segment pass to the celiac plexus of the lymph glands. For the most part, the efferent channels of the posterior mediastinal glands pass directly to the thoracic duct, while a few pass to the bronchial glands, which in turn drain into the thoracic duct. Among the afferent channels of the celiac plexus, besides those from the esophagus, are those from the hepatic, gastric, pancreaticosplenic, and lumbar nodes, while the efferents pass to the thoracic duct.

It is well known that carcinomata usually metastasize through the lymphatic system, yet there seem to be very good reasons for the belief that in some instances dissemination of the tumor tissue from the primary focus may be affected through the blood-vascular system, and in some instances it seems possible to take place over mucous or serous surfaces. Let us discuss the several cases seriatim.

Case I showed the carcinoma involving the cervical esophageal segment, but no metastatic growths were noted. Perforation of the trachea, however, had occurred. In Case II, unfortunately, the autopsy record failed to reveal the segment in which the primary tumor was found. There was widespread metastasis as before noted. Case III showed the tumor in the diaphragmatic portion of the tube, but no metastases had developed. Case IV showed the tumor in the lower portion of the tube as a very dense fibrous mass, but no metastases. Tuberculous laryngitis was a complication. Case V showed the growth in the lower segment of the esophagus, with the development of metastases to the pancreas, liver, and lungs. Explanatory of the abdominal and thoracic metastases it seems reasonable to suppose that dissemination occurred through the bloodvessels for two reasons—an absence of involvement of the celiac plexus of nodes and the finding of emboli composed of masses of squamous epithelium in the bloodvessels of the lungs. The probability is that the esophageal veins were effective in distributing the cancerous material with the lungs as the primary seat of deposit. Destruction of pulmonary tissue by the tumor process would facilitate its transmission to the pancreas and liver by way of the arterial system. Case VI

showed the tumor in the upper portion of the esophagus, but no metastases were noted. Case VII showed the tumor in the thoracic segment of the organ, with metastatic deposits in the liver, kidneys, stomach, posterior mediastinal, bronchial, hepatic, and lumbar glands. Involvement of the mediastinal and bronchial glands was naturally direct. The primary tumor mass had extended to the root of the lungs. In the stomach it was only in the muscular coats. It seems most reasonable that in this case dissemination took place through the vascular system, with the root of the lungs as the probable point of entrance of the cancer tissue into the blood. The involvement of the hepatic and lumbar glands was probably secondary to the involvement of the organs drained by these glands.

Case VIII showed the cancer in the diaphragmatic segment of the esophagus, but there were metastases to the posterior mediastinal glands. It is evident that the tumor area was drained by the thoracic lymphatics. In Case IX the location of the cancer was not indicated upon the record. Metastatic deposits were found in the stomach, and are explainable possibly by continuity of structure. Case X showed the tumor in the thoracic portion of the esophagus, and secondary growths were found in the stomach, pancreas, and bronchial glands. The presence of the gastric tumor may be explained as above, while the tumor of the pancreas probably developed by contiguity of structure directly from the stomach. Unquestionably the posterior mediastinal glands were affected to allow extension to the bronchial glands.

The conditions herein brought forth appear sufficient to warrant the statement that the secondary tumors developed not only through the channels of the lymphatic system, but also through the blood-vascular system. It seems possible that metastases may also take place over mucous surfaces.

In the consideration of the present series of carcinomata relative to the frequency of the same, the results show that of 1720 deaths, 8 were associated with the esophageal tumor, or a frequency of 0.46 per cent. These embrace the autopsy records from 1897 to 1907. During this period all records were filed, and the percentage, therefore, is an accurate one. Another point revealed is the preponderance of the squamous-celled tumor over that of the columnar-celled type. But one case of the latter type of tumor was found during the above-mentioned

period. There is a presumption that the tumor of the esophagus with "epithelial pearls" does not metastasize as frequently as does that without "pearls." Probably the same condition is true in this instance that applies to the reason why columnar-celled cancers metastasize with greater facility than do squamous-celled cancers. A resemblance of the cells of the basal cellular type to those of the columnar-celled type of tumor is assumed, so that the spinal cellular type (with "pearls") would appear to be, of all malignant epithelial tumors, the least likely to metastasize. Probably the shape of the cells and a difficulty in adapting themselves to the lumina of the smaller radicles of the lymph and blood-vascular systems render dissemination more difficult. The development of secondary tumors appears not unusual in squamous-celled cancers of the esophagus.

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October 24, 1907.

**Purulent Cerebrospinal Meningitis Caused by the Typhoid Bacillus,
without the Usual Intestinal Lesions of Typhoid Fever.**

BY J. NORMAN HENRY, M.D., AND RANDLE C. ROSENBERGER, M.D.

WILLIAM W., colored, aged thirty-four years, a native of Virginia, a coal picker by occupation, was admitted to the Philadelphia General Hospital, to the service of Dr. F. P. Henry, on account of headache, dizziness, constipation, and fever. His father and mother are dead; causes unknown. His wife and one child are living and well. He had had the ordinary diseases of childhood; otherwise he has always been healthy. The present illness began six days before admission, with headache, dizziness, and vomiting. He went to bed the next day, and was delirious on the third day. Severe pain in back of head, and some pains in legs were his complaints. He had some fever, but no epistaxis. He passed fairly large quantities of urine.

On physical examination he was a large, muscular, well-nourished negro, who lay in bed with his head slightly retracted, very restless, groaning, and picking at the bedclothes. He was profoundly unconscious. The eyes showed marked congestion of bulbar and tarsal conjunctivæ, and slight ptosis of both lids. The eyes were drawn upward and to the left. The pupils were small, equal, but did not react to light. There was some mucopurulent discharge from the conjunctivæ. The tongue was coated, dry, and fissured. Sordes were on the lips and teeth; the breath was foul, but had no characteristic odor. The pulse was rapid, regular, and the tension high. The pulmonary resonance was good throughout. The breath sounds were loud and clear. A few moist rales were heard at the right base. The heart was normal. The abdomen was rigid, but no special areas of tenderness could be palpated. Liver dulness seemed to be normal. Spleen could not be palpated. The arms were flexed and held stiff; the legs were tossed about. The knee-jerks were about normal, the right slightly more prompt than the left. Plantar irritation caused the foot to be drawn up, and the toes to be flexed on both sides. The spine was rigid and slightly concave. The neck was rigid. Kernig's sign was present.

April 4. Lumbar puncture was performed and about 25 c.c. of a turbid fluid withdrawn, which, on standing, deposited a heavy purulent sediment.

April 5. Kernig's sign was still present; the arms were somewhat rigid. The knee-jerks were absent; plantar irritation elicited no response. Still profoundly unconscious. Lumbar puncture was again performed, 30 c.c. of turbid fluid withdrawn, and 3000 units of diphtheria antitoxin and 30 minims of lysol were injected into the canal. Hot packs seemed to have beneficial effects on convulsions, which were not so frequent or severe.

April 6. The condition grew worse. Examination of fluid from the spinal canal showed no diplococci of Weichselbaum, but an organism which looked like the typhoid bacillus. Leukocytes, 14,000; Widal suggestive. Pulse rapid, weak, and almost imperceptible. Urine analysis: Amber; flocculent; acid; 1030; hyaline casts; a few large amorphous urates. Died at 5.15 P.M.

The cerebrospinal fluid was cloudy, alkaline in reaction, and contained small coagula. Cystoscopic examination showed 96 per cent. polynuclear cells, 2 per cent. lymphocytes, and 2 per cent. hyaline cells. Shreds of fibrin were also present. Bacteriological examination showed numerous bacilli, intracellular and extracellular, principally in the polynuclear cells, though a few were also seen in the hyaline forms and lymphocytes. These organisms possessed the morphological and tinctorial properties of a typhoid-like organism.

Inoculations made into various culture media gave rise to a growth of an organism which resembled the typhoid bacillus: that is, grayish-white growth on agar, cloudiness in bouillon, slight acidulation without coagulation in milk, no gas production in lactose or saccharose media, no liquefaction of gelatin, and no indol production in cultures. This organism resembled in all particulars the bacteria observed in spreads, and was agglutinated in dilutions of 1 to 40 with a known typhoid serum and with the serum of the patient.

Five c.c. of blood was obtained (under aseptic precautions) from the vein of the arm and inoculated into 200 c.c. of bouillon. A growth was observed in twenty-four hours, and consisted of an extremely motile bacillus, Gram negative, and resembling in subcultures upon various media the organism obtained from the cerebrospinal fluid.

Agglutination was positive in dilutions of 1 to 40 with the patient's serum and also with a known typhoid serum. Inoculations of 2 c.c. of a forty-eight-hour bouillon culture into the subcutaneous tissue of a guinea-pig failed to produce any pyogenic process.

At autopsy spreads and inoculations were made from the pus upon the brain. The spreads contained an organism identical with that observed in the cerebrospinal fluid during the life of the patient. A Gram negative motile bacillus was obtained which corresponded in all particulars with the organism isolated from the blood during life. Sections of the cord and cerebellum were stained with Loeffler's methylene blue and eosin, polychrome blue and eosin, and by the Gram-Weigert technique. Numerous intracellular and extracellular organisms were found, resembling the bacilli encountered in spreads and cultures; they were especially abundant in the cord.

This organism, from its morphological, tinctorial, and biological character, resembled in all particulars the *Bacillus typhosus*.

Certain of the autopsy notes (made by Dr. A. J. Smith) are of particular interest: The spleen weighs 110 grams, and is not adherent; capsule is smooth and thin; the organ is of a dark-slate color, rather flaccid, and cuts with normal resistance. Cut surface, dark red; Malpighian bodies enlarged; vessels and trabeculae not prominent. The intestines throughout are normal on the exterior; show venous congestion, and walls are quite thin. Mucous membrane of small intestine normal; toward ileocecal valve the membrane is injected, becoming red, and close to valve marked by points of hemorrhage. No follicular enlargement. Peyer's patches are large in their flat extent, but not raised; are pale and show no signs of typhoid medullary swelling or ulceration. Large intestine normal externally and in thickness of wall; likewise shows venous congestion. Mucous surface is moderately congested in upper part; shows no ulceration. Appendix 8 cm. long, of normal caliber, extends toward median line, and is adherent to posterior peritoneal surface. The lumen opened, the mucous surface shows follicles as tiny, black spots; no ulceration. Mesenteric glands are slightly enlarged, fleshy in consistency, reddened in color. Kidneys show nothing indicative of typhoid infection. Dura mater is tense and congested; upon removal the pial vessels are deeply injected and their course marked out by surrounding

yellowish lines of pus; no excess of meningeal fluid. At the base of the brain, about the pons and medulla, extending into the spinal cord, a large amount of pus wells out as in an abscess. Exposure of middle ear fails to show the presence of pus. Dura over base shows no evidence of extension of disease from nasal portion. Cord shows infection of vessels outside of dura throughout its extent, and, especially in upper two-thirds, it is covered with a purulent exudate, similar to that present upon the base and convexity of the brain.

Pathological Diagnosis. Purulent cerebrospinal meningitis; cloudy swelling of the liver and the kidneys; acute catarrhal enteritis, with enlargement of Peyer's patches.

The case reported is unusual because of presenting a purulent lesion caused by typhoid bacilli and because of the absence of the customary intestinal lesions of typhoid fever. The patient died on the ninth day of his illness and the autopsy showed that beyond a very little enlargement of the mesenteric glands and a slight change in Peyer's patches, which change might easily be accounted for by the enteritis which was present, the patient showed no typical typhoid lesions.

A bacillus in pure culture, which seems fairly identified as the bacillus of Eberth, was isolated from the blood and spinal fluid, from spreads and inoculations from the pus upon the brain, and was found in sections made from the cord and cerebellum.

Cole¹ reviews the literature of typhoid meningitis. He speaks of 14 cases reported by various authors in which there had been present purulent, fibrinopurulent, or hemorrhagic purulent meningitis, with general typhoid lesions. In 1 case, however, no autopsy was obtained. He also mentions 13 cases of similar purulent meningitis in which, however, the identification of the typhoid bacillus was not so certain, and of several cases in which there was mixed infection with other germs and the typhoid bacillus.

Neumann and Schaeffer report a case similar to ours in that there was present purulent cerebrospinal meningitis without the usual typhoid lesions, and though the organism isolated by them from the

¹ Johns Hopkins Hospital Reports for 1905.

pus appeared in many respects similar to the bacillus of Eberth, yet they did not feel altogether satisfied to place it definitely in that class.

MacCallum reviews at length the pathology of the condition, and finds "nothing peculiar in the histological study of typhoid meningitis unless it be the relative abundance of large phagocytic cells, found particularly about the veins, and also scattered through the tissues."

In our case there were a large number of polynuclear and mononuclear cells, but the large phagocytic cells did not predominate. In this instance the meningitis appears to have been a primary lesion due to the typhoid bacillus, and the intestines to have escaped the ordinary lesions which would be expected to be present at the ninth day of typhoid fever. The history was very carefully reviewed in regard to the possibility of the patient having had typhoid fever at a recent date, and the first statement that the man had been ill but six days before admission was firmly adhered to.

June 13, 1907.

Sarcoma Arising from the Thymus Gland in an Adult; an Associated Endothoracic Goitre.

By JOHN FUNKE, M.D.

THE material forming the basis of this paper is from an autopsy at the Philadelphia Hospital. The body was that of an adult male, aged forty-eight years. There was no evidence of the existing new-growth during the life of the individual, as there were no symptoms referable to such a condition; the clinical diagnosis was myocarditis and chronic interstitial nephritis.

At postmortem the lesions found were as follows: chronic endocarditis, emphysema (bilateral), pyelonephritis, suppurative ureteritis, suppurative cystitis, hypertrophy of the prostate, mediastinal tumor (sarcoma arising from an ectopic goitre).

When the thorax was opened there was present in addition to the pericardium with its contained heart another structure that looked not unlike a duplicate of the heart. This foreign mass lay on and along the pericardium, being loosely attached to it and extending from the upper

border of the third right costal cartilage to the lower border of the fifth; the greater portion of the mass was retrosternal. It measured 7 by 5 by 4 cm.; it was not firm, was reddish pink in color, presented a comparatively smooth external surface, and contained several cysts which were filled with a dark yellow substance; these cysts were not entirely fluctuating. Incision showed that the mass was encapsulated; the cut surfaces were reddish brown, and resembled those of a parenchymatous goitre. The centre of the mass was occupied by a semisolid substance; the peripheries were more firm, although not indurated. The cysts contained a semisolid gelatinous substance not unlike colloid. They were situated principally at the upper pole of the mass, from which point a fibrous band projected as far as the upper border of the sternum, where it gradually merged with the surrounding tissue; the band was not connected with the thyroid, which organ occupied its normal position and was not altered. The fibrous prolongation led to the belief that the mass was, perhaps, a part of the thyroid gland.

The diagnosis was, of course, not clear, but the growth was termed a mediastinal tumor, probably a sarcoma arising from an ectopic thyroid.

HISTOLOGY. The microscopic examination reveals the fact that the tumor is composed of two distinct parts; sections designated group 1 contain principally thyroid tissue; sections in group 2 contain typical sarcomatous tissue but no thyroid structure.

Sections in group 1 are largely composed of nearly normal thyroid tissue; there are acini present, the diameter of which reaches 0.75 cm., and which are either partially or completely filled with colloid substance containing many vacuoles. The stroma in many places contains, in addition to the acini, collections of epithelial cells the character of which is identical with those of the acini. Although these collections of cells are intimately associated with one another, close study shows that each is surrounded by a delicate strand of fibrous tissue, but, unlike the typical acini, they contain no colloid substance. Woelfler holds that such collections of cells form the basis from which tumors develop.

In one part of these sections is a gland-like area distinctly circumscribed by a delicate fibrous-tissue band. The acini-like structures

of which the area is composed are nearly uniform in diameter, and are lined by a single layer of epithelial cells which rests upon a very thin strand of fibrous tissue. In but one section do these acini-like structures contain colloid substance. About the centre of this particular gland-like area are five acini which are separated from the surrounding structure by fibrous tissue and are about to coalesce, the outlines of each being almost obliterated.

Along one margin of sections of group 1 are a few cells like those constituting the greater part of sections of group 2.

The last-named sections contain a band of dense, wavy, fibrous tissue in which there are but few cells, except at a few points where this band contains and surrounds collections of what are undoubtedly lymphoid cells. Usually along one margin, but now and then within the fibrous-tissue band, are masses of other cells constituting the greater part of the sections. Among these cells are bloodvessels and fibrous-tissue trabeculae, the latter arising from the broad band already mentioned. The elements of the cellular mass are, as a rule, large, round, or oval and closely packed. The protoplasm is not scanty and is not uniform in density; the perinuclear portion of the protoplasm is so rarefied that spaces appear to exist at these points, while the periphery of the protoplasm is more dense, but not granular; with Mallory's reticulum stain it takes a bluish tinge, and under low magnification seems to be an intercellular substance. The nuclei vary in size: some are circular, others are oval; some contain considerable, others little chromatin. For the most part there is no definite arrangement of the cells; at a few points, however, they are placed at right angles to what appear to be bloodvessels in some, and in other instances to bear but little resemblance to such structures. At these points there are at least two, sometimes three or four, strata of cells arranged in this manner. Here the packing of the cells is very much closer; the protoplasm can scarcely be identified.

The structures enclosed by the vertically placed cells have peripheries made up of hyaline fibrous tissue, which encloses cells the nuclei of which stain poorly and are spindle-shaped; the protoplasm is slightly granular. Then, too, there are present fragments of cells, nuclei, and an occasional polymorphonuclear leukocyte. In other places the fibrous sheath encloses not only cells like those just described,

but also erythrocytes. Here the nucleated cells are closely packed and the nuclei are spindle shaped; occasionally these cells tend toward concentric arrangement. Now and then the vertically placed cells enclose structures which are composed of hyaline fibrous tissue only.

In the fibrous tissue principally, but also occasionally among the cellular elements, are cells the nature of which correspond to those first described by Henle in 1865, and later by Stilling, who termed them "chromophile," because of their affinity for the chrome salts.

The bloodvessels are present in considerable numbers; their walls are at times extremely thin, and their lumina are nearly always filled with erythrocytes, leukocytes, and occasionally tumor cells. Besides the vessels with definite walls there are large blood spaces with apparently no walls, allowing many erythrocytes to mingle with the tumor cells.

With regard to the diagnosis, one can say positively that sections in "group 1" are ectopic or accessory thyroid tissue, and in all probability the nodule in these sections is an adenoma. The diagnosis of the tissue in sections, "group 2," is clear, I believe, as to the nature of the tumor, which I hold as a sarcoma, but the source of this growth is somewhat obscure. I admit that the structures enclosed by the vertically placed tumor cells are not all typical corpuscles of Hassel, but some of them do, it appears to me, resemble those structures very closely. Then, too, the lymphoid tissue found in the dense fibrous band simulates thymus-gland tissue.

Dugeon is convinced that, if searched for, the thymus gland would be found in most adults. He states that the corpuscles of Hassel in the adult gland may be hyaline, granular, or calcareous. Virchow maintained that a persistent thymus may become hyperplastic, and later take on a malignant nature in the form of a lymphosarcoma. In a recent communication to the Philadelphia Pathological Society, A. J. Smith produced evidence in support of Afanassieff's view that the corpuscles of Hassel are not vestigial epithelial remnants, but that they develop from vascular endothelium, which, if true, would furnish another source for the development of sarcomata.

In 1849, Gairdner reported a tumor of the mediastinum which he said grew from the thymus gland; Steudener reported a similar growth.

He found what he termed thymus rests, which were composed of small, round lymphoid cells. The growth was the size of an apple, and he maintained it was a hemorrhagic, small, round-celled sarcoma. Sir Astley Cooper, in his work on *The Anatomy of the Thymus Gland*, writes of carcinomata of this organ. Oser, Hedenius, Bramwell, Bienwalt, and also Hahn and Thomas report sarcomata arising from the thymus. Oser's case was a lad aged nineteen years, and Bienwalt's was a woman aged twenty-five years. The authors who mention the fact at all state that the corpuscles of Hassel were not present. Friedleben maintains that these structures are never present after twenty years.

It is not an infrequent occurrence to find thyroid tissue in the superior mediastinum. Richardson holds that just as the middle portion of the thyroid gland may form a pyramidal lobe above the lateral lobe, so in the same manner a pyramidal lobe may grow downward which may later separate from the thyroid and constitute an endothoracic thyroid. Paltauf, in reporting a case of intratracheal goitre, maintained that the ectopic tissue reached the walls of the trachea by direct extension from the normal thyroid or from the parathyroids. Cohnheim, Hollis, and recently Oberfeld and Steinhäus have reported cases of metastasis of thyroid tissue; the metastatic growth mentioned by the last named-authors, after removal, returned a year later, and then the patient died greatly emaciated. They hold that the microscopic pictures of the metastatic growths were identical with the normal gland.

It is, of course, possible that the tumor in the case reported in this paper arose from the ectopic goitre, but then I cannot account for the collections of lymphoid cells and the peculiar bodies surrounded by the tumor cells.

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Sarcoma of the Eyelid, with the Report of a Case in an Infant Seven Weeks Old.

By HENRY S. WIEDER, M.D.

(From the Laboratory of Surgical Pathology of the Medico-Chirurgical College of Philadelphia).

THE case which I wish to bring to the attention of the Society is of peculiar interest for several reasons: (1) Because of the tender age of the patient; (2) because of the situation of the growth; (3) because of the peculiar microscopic picture which the growth presented.

The patient, Albert H., an apparently healthy child, was noticed, when about seven weeks old, to be developing a small growth on his lower right eyelid. This growth, which was very small at first, grew gradually until it attained the size of a small pea. Two months after the first appearance of the growth the child was brought by his physician to see Dr. L. Webster Fox, to whom I am indebted for the privilege of reporting this case. Dr. Fox informed me that on examination the growth had all the earmarks of a simple lipoma, which was the diagnosis made at the time.

On February 27, 1907, a small incision was made over the growth

sufficiently large to introduce a pair of long, narrow forceps, which were inserted under the skin until they completely encircled the growth. It was then crushed between the blades of the forceps and brought out of the wound by a twisting motion. Even after the removal of the growth there was nothing about it to cause a suspicion of malignancy, it being soft and rather light in color. Its nature could not be studied grossly owing to the crushing it had sustained. As a matter of routine, however, it was sent to the Laboratory of Surgical Pathology for examination.

Gross examination was very unsatisfactory owing to the crushed condition of the specimen, so it was fixed and embedded in paraffin and sectioned.

Microscopic examination revealed a somewhat varied picture according to the portion of the tumor examined. Portions of the tumor were quite compact and cellular, while other parts appeared to be riddled with spaces which were apparently fat globules before fixation. The entire tumor was divided into lobules, many of which were separated from one another by a very delicate sheet of connective tissue, others by very thin-walled bloodvessels.

The size and shape of the cells constituting the cellular portions of the tumor varied greatly. In portions of the tumor, especially toward the edges, the cells were spindle-shaped, and all followed the same direction, running parallel with the outlines of the particular lobule in which they were found. In the central portions of the mass the cells were of varying shapes, some being somewhat spindle, others more polygonal, and still others apparently round. The protoplasmic outlines were not very distinct, the protoplasm of the different cells seeming to a certain extent to fuse. There was no intercellular stroma between the individual cells.

Some portions of the tumor contained abundant blood channels having no definite walls except those composed of the cells of the tumor proper. In places there were some signs of interstitial hemorrhage, which was probably caused by operative insult to the tissues. There did not appear to be any melanotic deposit in any part of the tumor.

The portion of the tumor which appeals to me as of particular interest is that in which the sarcomatous cells are seen growing

around what appear to be fat spaces. This is significant as a manifestation either of spreading infiltration into the surrounding fatty tissues, or, what may be more likely in view of the lobulation of the tumor (not alveolation), an evidence that the tumor was originally a lipoma which had undergone sarcomatous degeneration and was still in the process of that change. The subsequent history, in which it is found that a recurrence appeared in two weeks, renders it improbable that healthy tissue had been invaded during the extirpation of the growth, but more likely that a small microscopic portion of the tumor had been left behind, which would, too, substantiate the view of the lipomatous origin of the growth.

In calling attention to this case, the extreme youth of the patient appears to me to be a most striking fact. While the most frequent neoplasm found, sarcoma in infants under one year of age is, nevertheless, rather rare. Petit, in reviewing 5329 cases occurring within six years in the surgical and ophthalmological wards of the Bordeaux Children's Hospital, found that sarcoma was present in 31 cases up to fifteen years, constituting 58 per cent. of the total number of cases, but of these, only one was under the age of eighteen months, or less than 0.02 per cent. Proportionately the greatest number were between the ages of eighteen months and five years, 11 being found as compared with 19 for the next ten years.

Of the sarcomata in the literature occurring within the first year of life, by far the greatest number are tumors of the kidney. Most of these tumors, according to Walker, are probably embryonal in nature, many, doubtless, from misplaced adrenal deposits. Although resembling sarcomata in many of their characteristics, they are not true sarcomata and will not be classified as such. When they are excluded from the list, the sarcomata occurring during the first year are found to occupy a very insignificant place among the maladies of that period.

After a fairly thorough search of the literature, I have been able to find but few examples of purely sarcomatous tumors affecting infants under one year of age. In 1901 Pepper reported a case of congenital lymphosarcoma of the liver, and stated that he was able to find but 5 similar cases in the literature reported by Heaton, Orr, Parker, de Ruyter, and Meisenbach.

Curtis reported a very rare case of congenital periosteal, round and

spindle celled sarcoma of the acromion process the size of a hen's egg. While sarcomata in bones play a very important role among the sarcomata in childhood, Petit finding 15 osteosarcomata among the 31 cases he studied, I have records of only two occurring within the first year. Curtis's case is also one of the few showing any tumor formation of considerable size at birth.

Fisk, in 1896, reported a case of congenital growth of small size situated in the interscapular space, which, when removed, developed a recurrence which rapidly became general. Coley's fluid was used without effect, the child dying within a very short time.

Weinlechner reported 2 cases of congenital sarcoma, 1 a fibrosarcoma of the subcutaneous tissues over the scapula and the other a sarcoma of the parotid and sternomastoid. The only other example of congenital sarcoma that I have been able to find is that reported by Mandillon, who, at four days, found a mixed cell sarcoma of the right shoulder the size of an orange.

Marshall reported a very interesting case of spindle cell sarcoma situated between the superficial and deep muscles of the calf. The tumor was first noticed a fortnight after birth, from which time it appeared to grow rapidly until twice the size of the other leg, causing bowing forward of the tibia and fibula.

Jacobi reported an exceedingly rare case of sarcoma of the cutis discovered one month after birth. He quotes Neuhaus' case, in an infant five days old, and Karewski's case, in an infant seventeen weeks old, but claims that they are not identical with his and are not true sarcomata cutis.

Battle observed a case of melanotic, alveolar sarcoma in a child of six weeks originating at the site of the upper left incisors; and de Beurman and Gougerot, a case of multiple subcutaneous round cell sarcoma in an infant appearing at two months of age.

Carr reported a round cell sarcoma of the back at three months; Carpenter, a sarcoma of the dura mater in a child four months old; and Stowell, a fibrosarcoma of the liver at nine and one-half months of age.

The case described by Clark, occurring in the testicle, is of a rather indefinite nature, and is probably some form of embryonal rest tumor. Of the sarcomata affecting the eyelids in infants under one year, I

have found but three, *viz.*, those reported by Wood, at six weeks; Samelson, in a child of ten months; and Jacobi, a congenital angio-sarcoma.

From the above reports it would appear that sarcomata of the eyelid are not comparatively so rare in infancy as in later life, the cases mentioned here constituting 20 per cent. of the infantile sarcomata collected. I believe, however, that this is more apparent than real because I feel that I have overlooked sarcomata in other regions of the body because of their varied forms of classification, whereas I do not believe that I have missed many, if any, reports of sarcoma affecting the eyelid.

As a final reference to sarcoma in the first year of life, I would call attention to the fact that after the kidneys, the liver appears to be the most frequent site, constituting 31.5 per cent. of the cases in this list, while the region of the shoulder and back include 21.1 per cent. of the total. The majority of the growths were of the round cell or the mixed round and spindle cell variety.

When we consider our case from the standpoint of its situation, we find that sarcoma of the eyelid is a rather rare condition, a very careful search of the literature revealing but 44 cases, including the one reported in this paper. My records of some are incomplete, because the original articles were not available or the reports were not complete.

Of the 28 cases in which I have the age incidence, the first decade appears to be most frequently affected, there being 8 from birth to ten years, or 28.5 per cent. of the total number. Of these 8, 4 were found in the first year, or 14.3 per cent. of the total. When those occurring to the age of twenty years are compared with the whole number, they constitute 42.8 per cent. After the twentieth year, they are evenly distributed in the different decades until the seventy-sixth year, which is the oldest recorded case. It will be seen from the above that, although it is found at all ages, the affection is principally one of early life.

In the 31 cases in which I have a record of the region affected, the upper lid is the seat of the disease in 54.8 per cent., the lower lid in 29 per cent., all four lids in 6.5 per cent., the inner canthus in 6.5 per cent., and the outer canthus in 3.2 per cent. Of 22 cases, 12 were males and 10 females.

In 32 cases the nature of the cells composing the growth was obtainable, and averaged as follows: Round cells, 37.5 per cent.; spindle cells, 31.2 per cent.; mixed, round, and spindle cells, 15.6 per cent.; fibrosarcomata, 9.6 per cent.; giant-cell sarcomata, 6.1 per cent. Of 37 tumors, 29.7 per cent. were melanotic, 10.8 per cent. cylindromatous, and 5.4 per cent. angiomatous in nature.

It is interesting to note that of the 4 cases in which trauma was distinctly mentioned as a causative factor, all were upon the upper eyelid and all occurred between the ages of four and fourteen years.

In concluding, I wish to call attention to the final results in the case reported in this paper, which, taken in conjunction with a number of others reported by Dr. Pfahler, appears to present a possibility of more favorable prognosis in a condition which has always been considered as offering but little hope of cure. As stated in another portion of this paper, two weeks after removal of the growth a recurrence was noted nearly equal in size to the original tumor. Treatment by the Röntgen rays was then begun, and after five months' treatment the growth entirely disappeared. The child has since remained entirely healthy and free of any signs of tumor formation, a lapse of nine months since the time of the original operation.

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Present Status of the Etiology of Syphilis. The *Spirocheta Pallida*, its Biology and Etiological Relation to the Disease.

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PREVIOUS to the discovery of the *spirocheta pallida* the lesions of syphilis were seemingly successfully produced in the lower animals, by inoculation with human virus, by quite a number of observers, among whom may be mentioned Metchnikoff and Roux, Martineau, Adrian, Hugel, Holzhauser and Neisser (quoted by Flexner, *loc. cit.*).

That the disease could be transmitted was proved especially in monkeys, but far better in anthropoid apes. From the lesions

produced, there seemed to be no specific organism isolated. Numerous organisms and so-called parasites were recognized, and especially among these apparently specific parasites was the cytorrhycles luis of Siegel (*Münch. med. Woch.*, 1905, lii, 1323, 1384, 1574), a so-called protozoön, which he described as occurring in the blood and tissues of syphilitics and in rabbits inoculated with the blood and other syphilitic products. This was followed by the work of Schaudinn and Hoffman upon syphilis, with the result that they observed an organism constantly in the lesions of syphilis which they termed the *S. pallida*.

The constancy with which the *S. pallida* is found in primary and secondary lesions of syphilis seems to strengthen the assertion of Schaudinn and Hoffman, that it is the probable cause of the disease. The only remaining step seemingly necessary to complete this assertion is the cultivation of the organism upon some culture medium. It has been found in the lesions; it has produced lesions when inoculated into numerous animals (apes, monkeys, sheep, dogs, rabbits), and has been recovered from these lesions, and agglutination reactions have also been recorded by some few observers.

MORPHOLOGY OF *S. PALLIDA*.—Schaudinn and Hoffman (*Arbeiten aus dem Kaiserlichen Gesundheitsamte*, 1905, xxii, 2, 527, and *ibid.*, *Berl. klin. Woch.*, 1905, 22) first describe two forms of spirochetæ occurring in syphilitic lesions not only in superficial, but also in deep lesions, and in the connected lymph glands.

The *S. pallida* is delicate, 4 to 10 μ in length, average length 7 μ , its breadth about 0.25 μ . The spirals number 6 to 14, are abrupt, narrow, regular, and deep. It has pointed ends, progresses by rotating upon its long axis, and when at rest shows undulatory movements in its whole length, suggestive of the play of a vibratile membrane. It stains with difficulty, and with Giemsa's stain takes on a light purple hue. Schaudinn later called the organism the *treponema pallidum*.

The *Spirocheta refringens*, the second form of spirochetæ observed by Schaudinn and Hoffmann, differs from the other spirocheta in that it is slightly wider and the spirals are farther apart, and it stains more intensely with the dye. The latter organism is found in other conditions than syphilis.

Vuillemin (*Compt. Rendus de l'Acad. des Sci.*, 1905, cxi, 23, 1567) proposed the term "spironema" for spirochetæ with sharp ends. The *S. pallida* would thus become the "spironema pallidum."

Some observers thought that the organisms *S. refringens* and *S. pallida* were simply stages of one organism, but this was before the demonstration of the parasite (*S. pallida*) in the blood. Among these may be mentioned Jesionek (*Münch. med. Woch.*, 1905, 49, 2394), Kiolomeneglon and von Cube (*Münch. med. Woch.*, 1905, 27).

According to Pfender (*Amer. Med.*, March 10, 1907, p. 350) the generic name, spirochete Cohn, 1872, simply represents an amended spelling of spirocheta Ehrenberg, 1834. As the name spironema is preoccupied in zoölogy, the present correct name, under the International Code, should be treponema pallidum. Stiles and Pfender (*Amer. Med.*, December 2, 1905) also proposed the term microspironema.

ANIMAL OR VEGETABLE? Minchin (*Lancet*, September 7, 1907, p. 707) mentions that the question of considering the spirochetæ among the hemoflagellates and not among bacteria is still being debated, but the weight of evidence is now toward the belief that they are really protozoa.

Mott (*Lancet*, September 7, 1907, p. 712), in exhibiting specimens of gummata of the brain, mentioned the fact that syphilis "is coming to be regarded as due to one form of protozoön, the *S. pallida*."

Schaudinn (*Deut. med. Woch.*, 1905, p. 1665), Hexheimer, and Löser (*Münch. med. Woch.*, 1905, p. 2212), with Loeffler's flagella stain, have demonstrated flagella upon the *S. pallida*. The latter observers also succeeded with ordinary dyes.

Thesing (*Sitzungsberichte der Gesellschaft. Natur forschender Freunde*, Jahrgang, 1905, Nr. 8, 9) disputes the propriety of classifying the spirochetæ among the protozoa, and denies that the significance of *S. pallida* as the cause of syphilis has been proved.

Norris, Pappenheimer, and Flournoy (*Jour. Inf. Dis.*, May, 1906) claim that the absence of any definite indication of long division and the absence of such chromatic particles as justify belief in the existence of micro- and macronucleus; the unquestionable occurrence of transverse fission; the positive evidence of production of active immu-

nity, and, as shown by Novy, the formation of antibodies in sufficient amount to lend passive immunity; these facts indicate that the spirochetæ must not be considered as protozoa. Goldhorn (*Jour. Exp. Med.*, 1906, 8, 451) also believes in the transverse division of the parasite.

Schaudinn places *S. pallida* with the protozoa rather than with spirillar bacteria, allying it to the trypanosome group.

M. Schuller (*Cent. f. Bakt.*, 1905, xxxvi, 24, 25) criticises the technique employed by Schaudinn, and cannot understand Schaudinn's grounds for placing spirochetæ among animal parasites.

Schaudinn (*loc. cit.*) claims that the parasite may possess, besides flagella, a vibratile membrane, though this is not yet clearly demonstrable. As these characters separate *S. pallida* not only from spirochetæ and spirilla, but from all other related forms, he adopted at one time the name *spironema* (of Vuillemin).

Hexheimer and Löser (*Munch. med. Woch.*, 1905, 46, 2212) describe the presence of granules in the *S. pallida*, possibly of the nature of blepharoblasts, also small free bodies with nucleus, protoplasm, and membrane, possibly representing a developmental stage. They think the appearances formerly described as a vibratile membrane were artefacts, and confirm the presence of flagella.

Dalous (*Jour. Mal. Cut. et Syph.*, 1905, xvii, 481), with a quartz lens and magnification of from 2500 to 4000 diameters, claims that an undulating membrane can be demonstrated.

Wechselmann and Loewenthal (*Med. Klinik.*, Berlin, June 4, vol. i, 26) report the appearance of a nucleus in quite a number of specimens examined with an ultramicroscope.

Doflein (Fourteenth Intern. Congress of Hyg. and Demog., *Brit. Med. Jour.*, October 19, 1907, p. 1075) regards the spirochetæ as taking a position midway between the bacteria and protozoa, and prefers to speak of them as proflagellates. They multiplied partly by transverse and partly by longitudinal division; the former method was best marked in the smaller forms; in some, what he regarded as multiple division could be seen, and no sexual processes were noted.

Levaditi (*ibid.*) claimed that many of the spirochetæ had no distinguishable morphological characteristics.

Manson and Sambon (*Med. Record*, October 5, 1907, p. 586), in a table upon the hemoprotozoa, classify three genera under spirochaudinniidae (the individuals characterized by elongating and breaking up into numerous sporozoites), the leukocytozoön, spirochaudinnia and the treponema.

Krzyształowicz and Siedlecki (*Bull. Ac. Sci. Cracovie*, November, 1905, p. 713 to 728, No. 9) claim that the body of the *S. pallida* is contractile, and can become, thereby, much shorter and thicker, with its curves less sharp and at the same time more refringent; but the pointed extremities remain a distinctive feature. At some point, generally not far from the middle of the body, it is seen that for a short distance the body is straight or nearly so, and very slightly thickened, and in this region a clear spot can be observed which is regarded as the nucleus. The ordinary method of reproduction is by fission in a longitudinal direction. The fission may, for a time, stop short of completeness, with the result that the two sister individuals may remain connected by their ends. In addition to the ordinary individuals, they also describe forms which they consider as sexual individuals. These are thick, spindle-shaped forms with few bends, which the authors consider trypanosome-like, and propose to name trypanosoma luis, although the minuteness of the organism makes it impossible to identify the undulating membrane; secondly, minute spirillum-like forms with several nuclei. The former are regarded as macrogametes derived each by growth of single treponema-individuals; the latter as microgametes formed by a process akin to sporulation from an individual with multiple nuclei. Conjugation between the two forms was noticed in a single case in "materials taken from a very large primary ulceration which was beginning to cicatrize spontaneously."

Kreibich (*Wiener klin. Woch.*, 1907, Nr. 21), in staining flagella of bacteria with silver solutions, noticed attached flagella which were often spiral and twisted together like braid, and, being pointed at both ends, reminded him very much, in appearance, of the *S. pallida*. He then questions whether, on account of this similarity, the *S. pallida* may not be a protozoön flagellum instead of a difficultly stained individual bacterium, especially because in the spirocheta differentiation between nucleus and membrane has not been

established. Kreibich answers his own question by saying that the *S. pallida* is unlikely to be identical with a flagellum, because no protozoan body from which the latter might have come has yet been discovered.

OCCURRENCE OF THE *S. PALLIDA* IN THE LESIONS OF SYPHILIS.—Mulzer (*Berl. klin. Woch.*, September 4, 1905) claims that the *S. pallida* is almost always found in the products of infectious syphilis, and has never been observed in the healthy or in non-syphilitic persons.

Levaditi and Petresco (*Presse Méd.*, September 30, 1907) blistered three syphilitic women, and found the parasite in the serum of each case.

Levaditi and Salmon (*Le Semaine Méd.*, November 29, 1905, No. 48) found the organism generally distributed through all the organs, as the lungs, adrenals, liver, skin, etc. (indicating an acute spirillosis), in a still-born child. The disposition of the *S. pallida* in groups around the vessels in the liver seems to favor the theory of the penetration of the microörganism by the vascular route.

Scholtz (*Deut. med. Woch.*, September 14, 1905) found the *S. pallida* in four cases of syphilis comprising condyloma acuminata.

Burnet and Vincent (*La Sem. Méd.*, November 29, 1905, No. 48), in recent syphilitic chancre (four or five days), found the organism abundant in the papillary layer of the adjacent skin, but rarely in the central part. They were also successful in demonstrating the parasite in the hypertrophied conjunctival layer and in the lymph spaces.

Levaditi and Manouelian (*ibid.*) claim that, in a study of a number of chancres and syphilitic papules, in all they were able to find the *S. pallida* in the tissues and bloodvessels and endothelial cells, undoubtedly playing a part in the production of periarteritis and the characteristic lesion or chancre.

Veillon and Girard (*La Sem. Méd.*, December 27, 1905, No. 52) assert that the roseola is not a toxic lesion, but is caused by a true embolism of the parasite which, transported by the blood, becomes fixed in the terminal capillaries, where it provokes an intense congestion.

Tchlenoff (*Roussky Vratch*, June 18, 1905) studied 14 cases of syphilis in which he was able to demonstrate the *S. pallida* in all instances in the secretion of chancres and inguinal glands. In all

cases of hard chancres he found spirochetæ in the specimens, and also in the moist papules, as well as a papule upon the tongue.

Sobernheim (*Münch. med. Woch.*, September 26, lii, Nr. 39) observed the *S. pallida* in 50 out of 58 cases of certain syphilis, the positive cases being those with primary or secondary lesions, while the 8 negative cases were tertiary. In 34 control cases of various affections the findings were invariably negative.

Schlimpert (*Deut. med. Woch.*, 1906, p. 1452, Nr. 36) found the *S. pallida* in the conjunctiva, sclerotic, cornea, iris, choroid, and in the muscles of the eye and lacrymal sac. It was most frequently seen within the vessels and free in the blood.

Schütz (*Münch. med. Woch.*, liii, Nr. 12), in making comparative studies of Schaudinn's spirocheta pallidum and Siegel's cytorrhcytes luis, mentions the fact that both are always found together and close to or inside the red corpuscles. He thinks it is possible that they represent merely different phases in the life cycle of a single micro-organism.

Schor (*Roussky Vrach*, September 10, 1905) examined 25 adults and 7 children for the spirocheta of syphilis. In 25 women with condyloma the organism was demonstrable in 15; in 2 chancres, only 1 showed the parasite, and in 3 roseolous eruptions none showed a positive result. In 13 out of 14 cases of papular syphilide it was found; 5 cases of dry papules were negative. No organisms were found in the placenta of 5 women with condyloma, and in 4 infants no organisms were found in any of the organs.

Ravant and Ponselli (*Gaz. des Hôp.*, July 13, 1906) found the parasite in the blood of a child, aged two months, suffering from severe congenital syphilis. The blood was taken two hours before death.

Gierke (*loc. cit.*) reports positive findings in 11 cases of congenital syphilis. He obtained positive findings in tissues that had been preserved for years, especially those kept in formol. His findings also prove that the treponema long resists the effects of maceration.

Nattan-Lanier and Bergeron (*Presse Méd.*, January 10, 1906) report 3 cases of syphilis in which the *S. pallida* was found in the blood.

Bandi (*Gazz. degli Ospedali*, Milan, 1906, xxvii, No. 51) calls attention to the importance of the accumulations of spirochetæ

inside the cells. According to him, it is not a phagocytic process, as the organisms seem to be intact, while the cell seems to have suffered. He believes that the condition indicates actual vital parasitism of the cell.

MacLennan (*Brit. Med. Jour.*, May 12, 1906) was able to demonstrate *S. pallida* in only 8 out of 40 cases of syphilis in the female, but found the cytorrhcytes luis in every case.

Sydney Stephenson (*Ophthalmoscope*, March, 1906) states that he found the *S. pallida* in the corneal lesions in 2 cases of keratomalakia in syphilitic infants.

Mohn (*Zeit. f. Geb. und Gyn.*, Stuttgart, lix, Nr. 2) found the pale spirocheta in more than 5 per cent. of umbilical cords examined, and in almost 70 per cent. of the placenta. The absence of nerves in the placenta shows that the organisms found cannot be explained away as "silver-stained nerve fibrils," as some have asserted in respect to other tissues. The fact that the placenta and membranes are free from external infection also excludes error from this source. They were never found in the decidua or intervillous spaces, but they swarmed in the fetal villi and in the umbilical cord when the fetus exhibited signs of syphilis—not otherwise. He further maintains, that the parasites find their way into the ovum from the mother or in the spermatie fluid, or pass from the mother to the fetus later. They proliferate in the fetus, acquiring new virulence, and pass thence into the placental circulation.

Jacquet and Sevin (*Annales de Dermatologie et de Syphilographie*, June, 1905) found the *S. pallida* in secondary lesions, but failed in 23 tertiary lesions.

Kraus and Prantschoff (*Wien. klin. Woch.*, 1905, 37, 941) found the *S. pallida* in 32 out of 37 hard chancres, and in 18 out of 25 papules. In the lesions of four macaque monkeys, *S. pallida* were found.

Mulzer (*Berl. klin. Woch.*, September 4, 1905) was successful in observing the parasite in 20 out of 22 cases; in 1 instance he found a mass of organisms of from 20 to 40 individuals. He was unable to find the parasite in smegma, or in patients with non-specific ailments.

Scholtz (*Deut. med. Woch.*, September, 1905, 37) doubts whether the *S. pallida* is of etiological significance. In primary and second-

ary lesions he had 19 positive and 11 negative results, and 2 positive and 1 negative result in congenital syphilis.

Rille and Vockerodt (*Münch. med. Woch.*, 1905, 34) found *S. pallida* in 22 different lesions in 14 syphilitics. They obtained negative results in roseolar blood and syphilis hemorrhagica neonatorum.

Sobernheim and Tomaszewski (*Münch. med. Woch.*, 1905, 39, 1857) were successful in 50 primary and secondary syphilitics in demonstrating the *S. pallida*, but were unsuccessful in 8 cases showing tertiary lesions.

In tertiary lesions Schaudinn (*Deut. med. Woch.*, October 19, 1905) observed the organism as a granular resting form. He also cites 70 cases of syphilis in which the organism was constantly found.

Siebert (*Deut. med. Woch.*, 1905, 41) obtained positive results in 52 out of 66 cases of primary, secondary, and congenital syphilis; negative in lesions not syphilitic, and in tertiary (7) lesions.

Roscher (*Berl. klin. Woch.*, 1905, 44, 45, 46), of 206 syphilitic lesions, obtained 184 positive and 22 negative results.

Zaboltny (*Roussky Vrach*, March 17, 1907) obtained fluid by means of an aspirator from indurated chancres in which he found *S. pallida*. The addition of physiological salt solution retains the organism in viability for several days. The presence of serum of those suffering from syphilis, when brought in contact with these organisms, causes agglutination, which phenomenon is complete within three or four hours. This fact, the author thinks, speaks more for the specificity of the *S. pallida*.

Pasini (*Giorn. Italiano delle Malattie ven. et d. pelle*, 1906, p. 4, f. 5), in a child, aged two and one-half years, suffering from hereditary syphilis, presented two resumptions of the disease. The first was characterized by exanthematous papules, and the second by an eruption of papules and a mucous patch on the palate. The child was treated by sublimated baths, by inunctions, and by injections. It died in two and one-half years of tuberculosis of the lungs, and did not present any acute lesions of syphilis after recovery from the second recurrence. On the left leg there was an atrophic lesion resulting from the first infection, and this was examined histologically by Bertavelli and Levaditi. Pasini found, in this lesion, numerous *S. pallida*, some intracellular, but for the most part extracellular, some

perfectly preserved, others in a degenerating condition. He concludes that in the treatment of this child, although apparent health had been restored, the *S. pallida* preserved itself during the long period of latency in the same tissues.

The same observer, in a later article (*Giorn. Italiano d. Mallat. ven. et d. pelle*, 1906, p. 5, f. 5), claims that the penetration of the epithelial cells by the *S. pallida* is not an agonizing phenomenon, but is followed by the phenomena of degeneration, and that the organism could well survive in the urine, saliva, and in the perspiration of children with hereditary syphilis, and in the sperm of the adult.

Wersilowa (*Central. f. Bakter.*, I. Origin., October, 1906, t. xlii, p. 513 to 518) claims that the transmission of syphilis to the child could be effected by the ovum and spermatazoa, sometimes by the placenta. He cites the history of three congenital syphilitics: The first one was macerated, the second lived only several hours, and the third died one hour after birth. The mother was apparently healthy. The first two presented plantar pemphigus and numerous papules, and the third did not present any cutaneous manifestations. Examination of spreads from the umbilical cord, heart, lungs, pemphigus, and papules revealed *S. pallida*, while sections showed numerous organisms in the placenta, umbilical cord, liver, heart, lungs, spleen, pemphigus, and papules. The author has made similar studies in 25 other cases of hereditary syphilis, but did not confirm the preceding declarations. Of all these examinations, he concludes that the parasite of syphilis could be transmitted to the mother by the placenta or umbilical cord, and that he could verify the spirocheta in the placenta and cord and the organs of the children without the mother presenting the least symptom of the malady.

Mühlens and Max Hartmann (*Central. f. Bakter.*, I. Origin., January 17, 1907, t. xliii) claim that the cytorrhcytes luis of Siegel is not a protozoön, and that similar bodies exist in the normal blood, and that these forms are to be considered as the products of disintegration of cells, especially the red blood cells.

Mühlens (*Central. f. Bakter.*, I. Origin., March 23, 1907, April 6, 1907, t. xliii) confirms the relation of *S. pallida* as the etiologic factor of syphilis. In 22 cases of primary lesions and in buboes he found it in a number of the cases. In 18 syphilitic fetuses he found the organism

constantly, even although the fetus was macerated. He also emphasizes the fact that the spirals obtained by Levaditi's method are true spirochetæ. He concludes by citing the case of a syphilitic infant, who died four hours after birth and was examined one hour after death. Examination of spreads of different organs—liver, adrenal, and lung—showed great numbers of motile spirochetæ, 50 to a microscopic field, in the smear from the adrenal.

Ribadeau-Dumas et Poisot (*C. R. Soc. Biologie*, February 16, 1907, t. lxii) in an infant presenting diffuse hepatitis, with hemorrhages and diffuse miliary gummata, showed uniform presence of spirocheta pallida. In the other parenchymatous organs the parasites were found to be in such numbers as to constitute emboli.

Bab (*loc. cit.*, *Zeit. f. Geburts. und Gyn.*), in 14 instances of congenital syphilis, claims that the biological and bacteriological investigations gave exactly the same result. Not only those organs that were free from spirochetæ gave extracts free from antigens, and organs containing the parasites gave extracts containing antigens, but also a great number of spirochetæ corresponded to a great number of antigens. The contrary was also true, that a small number of spirochetæ contained a small number of antigens.

The important problem presents itself, that the placenta acts like a sponge and stores up the dissolved syphilitic toxins, and the granulation tissue elements act as phagocytes which also absorb the dissolved toxins and by this process endeavor to make them inert. But it is possible that after a time the barrier becomes incompetent toward the mother or toward the child and becomes permeable to the toxins. The placenta seems to correspond to the fetal liver and spleen, which are sometimes swollen but contain no spirochetæ.

He examined 64 cases of syphilis, and found the spirochetæ most commonly situated in the vessel wall and in connective tissue. He was able to detect them in the spleen in 62.9 per cent., and in the thymus gland in 55.6 per cent. In the fetal ovary the interstitial tissue is especially affected, and one illustration accompanying his article shows a spirocheta in the ovum itself. This penetration into this structure proves distinctly that it cannot be nerve or other tissue fibers. In 39 cases examined, he found the placenta free from spirochetæ in 37. In general, the presence of the parasite seems to end at the navel,

while in 50 per cent. the arterial walls and in 55.6 per cent. the venous walls of the umbilical cord of his positive cases contained the organism. He also seems to think that one cannot avoid being impressed with the fact that the spirochetæ masses are disseminated through the body into different organs in the same manner as an embolus is carried by the blood. He then mentions the case of a congenitally syphilitic infant from a woman who had intercourse with a syphilitic at the fourth month after conception, although herself healthy. The thymus gland, kidneys, uterus, meninges, and eyes contained spirochetæ.

Buschke and W. Fischer (*Deut. med. Woch.*, 1906, xxxii, p. 752), Levaditi and Manouelian (*C. R. Soc. d. Biol.*, Paris, 1906,) Reischauer (*Deut. med. Woch.*, August 24, 1905, xxxiv), Oppenheim and Sachs (*Deut. med. Woch.*, 1905, Nrs. 29 and 31, and *Münch. med. Woch.*, 1905, 1507 and 1517), were also successful in obtaining positive results in the majority of cases studied. The latter observers found *S. pallida* in 39 hard chancres and papules, and failed in 21. They were unsuccessful in the examination of 9 buboes, 21 examinations of the blood, 15 roseolar spots, 2 mucous plaques, 7 gummata, and 4 cases of congenital syphilis. In 42 non-syphilitic conditions they found no spirochetæ pallida.

Bertarelli and Volpino (*Central. f. Bakt.*, I. Origin., November 24, 1905) found *S. pallida* in 26 cases out of 42 primary and secondary cases. By the use of Levaditi's method they demonstrated great numbers of the organisms in sections of the liver in a congenitally syphilitic child, and control observations showed that they had not to deal with elastic fibers, connective tissue fibrils, or nerve endings.

Rosenberger (*loc. cit.*), in 56 cases of primary and secondary syphilis, as well as the viscera of congenitally syphilitic infants, found the spirocheta pallida constantly. They were, as a rule, few in number in spreads, although in one chancre as many as 40 or 50 organisms could be seen. They rapidly decreased in number when mercury was given.

Uhle and MacKinney (*Jour. Amer. Med. Assoc.*, February 16, 1907) report upon 24 cases of acquired syphilis, in which positive findings were reported in 14 and negative in 10. In the patients giving a negative result, 7 had received antisyphilitic treatment, the other 3

had not. They examined 34 pieces of tissue in all, including 7 chancres, 19 cutaneous secondary lesions, apparently healthy skin from syphilitics, 4 tuberculous syphilides, and 1 gumma of the brain.

Richards and Hunt (*Lancet*, September 30, 1905) found the *S. pallida* in 3 cases in the blood taken from the roseolar rash on each of ten successive days.

Dudgeon (*Lancet*, March 10, 1906) observed the *S. pallida* in 6 cases of primary syphilis, 2 cases of secondary syphilis, 1 case of tertiary syphilis, and 3 cases of congenital syphilis.

Shennan (*Lancet*, March 17, 1906) precedes his personal research with an excellent bibliography of the subject. He records 5 hard chancres with positive results; in 2 the results were doubtful, and in 3 negative. Two non-ulcerated papules gave positive results, 2 dry papulosquamous syphilides gave negative results, and 2 roseolar rashes were also negative. Of 4 condylomas examined, 3 were negative and 1 doubtful. Negative results were also obtained in 4 glands and 1 rupioid syphilide. He concludes that the *S. pallida* is found only in hard chancres and in closed papules; in other words, in typical syphilitic lesions.

Flexner (*loc. cit.*) gives the results of several cases of acquired syphilis and also of congenital syphilis in which he obtained positive and constant results in all cases. In the case of films made from lesions, he was able to demonstrate the parasite several months afterward. Fragments of lung tissue, kept in the refrigerator (2° to 4.5° C.) for one month, showed very little, if any, change in the form and staining of *S. pallida*, while, after three months, no *pallida* could be stained. The slow autolysis, without putrefaction, shows the resistance displayed by the organism against the autolytic ferments.

Grouven and Fabry (*Deut. med. Woch.*, Leipsic and Berlin, September 14, vol. xxxi, 37) observed the *S. pallida* in 15 out of 21 cases.

Kraus and Prantscoff (*Wien. klin. Woch.*, June 21, vol. xviii, 22) found the parasite in 50 out of 62 examinations of syphilitics.

Schultz (*Jour. Med. Res.*, 1906, vol. xv, p. 363), reporting upon the distribution of the *S. pallida* in 2 cases of congenital syphilis, says that it is, to a marked degree, an intracellular parasite, and that multiplication of the organism occurs chiefly in the perivascular

lymphatics and within the tissues themselves; not within the larger bloodvessels.

Wiens (*Arch. f. Ach. und Tropenhyg.*, 1906, t. x, Nr. 15, pp. 459 to 463) reports 6 cases of syphilis, in Chinese, in which he was able to demonstrate the *S. pallida*. In 4 cases he obtained it from chancres, in 1 from serum of a roseolous eruption, and in 1 case from juice from an inguinal gland.

Bab (*Deut. med. Woch.*, November 29, 1906, pp. 1945 to 1948) records finding the parasite in the different structures of the eye, except the crystalline lens, in a syphilitic fetus. He thinks that the distribution of the organism, in the different parts of the eye, is in accord with the frequency of choroiditis, iritis, and parenchymatous keratitis in hereditary syphilis.

Menietrier and Duval describe a septicemic form of hereditary syphilis evidenced by the presence of the *S. pallida* in the blood and all the viscera, accompanied by congestive lesions. In the liver the congestion attains an extreme degree, recalling the characters of the "asystolic liver."

Reuter (*Zeit. f. Hyg. und Infekt.*, 1906, 49) found typical examples of *S. pallida* in the tunica intima of the aorta, in a case of aortitis (Döhle and Heller's variety of aortitis), and also mentions finding the organism in a gumma of the lung, and in interstitial pancreatitis of a congenital syphilitic.

Neoggerath and Stahelin (*Münch. med. Woch.*, August 1, 1905) claim to have observed the *S. pallida* in the blood, obtained from the lobe of the ear in 3 cases of undoubted secondary syphilis.

Risso and Cipollino (*Ref. Med.*, August 26, 1905), in 10 cases of syphilis, were successful in demonstrating the *S. pallida* in 5 out of 7 cases in the gland juice; also in several mucous patches and 1 condyloma. They failed, however, to find the organism in 2 chancres, an ulcerated gummata, and in a closed gumma of the forehead.

TECHNIQUE.—In spreads from chancres, papules, and other syphilitic lesions, Schaudinn and Hoffmann originally recommended Giemsa's azure-blue eosin stain, but very good results have been obtained with Wright's, Jenner's, Romanowsky's, Leishmann's, and Goldhorn's stains. Many observers recommend various dyes,

a filtered saturated aqueous solution of gentian violet (Fox) and other modifications of the various blood stains.

Among those who recommend the aniline dyes may be mentioned Davidsohn (*Berl. klin. Woch.*, July 31, vol. xlii, 31); Rille and Vocke; Rodt (*Münch. med. Woch.*, August 22, vol. lii, 34, p. 1620 to 1623); Ploeger (*Münch. med. Woch.*, July 18, vol. lii, 29); Oppenheim and Sachs (*Deut. med. Woch.*, Leipsic and Berlin, July 20, vol. xxxi); Hexheimer (*Münch. med. Woch.*, 1905, 39); Bandi and Simonelli (*Gazz. de Ospedali e delle Cliniche, Mil.*, 1905, 85 and 105); Moncorvo (*Presse Méd.*, Paris, 1905, 104 to 840); Dudgeon (*Lancet*, August 19, 1905, p. 522); and Weitlauer (*Münch. med. Woch.*, 1905, 47, 2293).

In sections of tissue from syphilitics, it seems that the best results are obtained by impregnating with nitrate of silver in strengths of from 1 to 3 per cent. The first investigators to use this method were Bertarelli and Volpino (*Cent. f. Bakt.*, I. Origin., November 24, 1905). Since these observers used the method, numerous others have obtained very good results, among whom may be mentioned Buschke and Fischer (*Berl. klin. Woch.*, 1906, i, 6), Gierke (*Münch. med. Woch.*, 1906, liii, Nr. 9), and Levaditi (*Annales Past. Inst.*, January, 1906, p. 43, No. 1). The latter's technique, together with one which he and Manouelian (*C. R. de la Soc. Biol.*, 1906, lx, No. 3) originated, are by far the best for the demonstration of the *S. pallida* in the tissues. The first technique is as follows:

The tissue is cut in small masses and fixed for twenty-four hours in a 10 per cent. solution of formalin; then placed in alcohol for the same time. They are then washed in water for a short period, after which they are put in a bath of 1.5 to 3 per cent. freshly made solution of nitrate of silver for three days, changing the solution daily, maintaining the body temperature and excluding light. The tissue is then placed in a reducing bath which consists of a 2 per cent. solution of pyrogallie acid, with the addition of 5 per cent. formalin. After twenty-four hours they are dehydrated, cleaned in xylol, and embedded in paraffin.

The method recommended by Levaditi and Manouelian differs from the plain Levaditi method in that, just before impregnation with the silver, they add 10 per cent. pyridine to this solution, and for the reducing bath a mixture of pyrogallie acid, acetone, and pyridine.

In the silver solution the tissues are kept four to six hours at 50° C., or at room temperature two to three hours in glass stoppered bottles.

Flexner (*Jour. Exp. Med.*, July, 1907, vol. ix, No. 4) prefers and obtains the best results in films by the use of the direct silver staining, recommending the technique of Stern (*Berl. klin. Woch.*, 1907, lxiv, 400) which is as follows:

The exudation is placed in the incubator at 37° C. for one hour, then in a 10 per cent. solution of silver nitrate for one hour in diffuse daylight. A colorless glass vessel should be used. The preparation gradually takes on a brown color, and when it has acquired a metallic sheen it is removed from the silver nitrate solution and washed in water. In such a preparation the form of the blood corpuscles is retained; they give a strong, dark contour and show fine granules.

There is but little precipitate, and it causes no annoyance in looking for the organism. The *S. pallida* appear deep black to bright brown against an almost colorless background. A reduction of the preparation is neither necessary nor advisable. Placing it in the sunlight gives the material a brown color, which becomes gray-black from a quarter to half an hour later, and finally entirely black. The *S. pallida* appear almost colorless against a dark background.

Schmorl (*Deut. med. Woch.*, 1907, xxxiii, 876), fixes in 4 per cent. formalin. Cuts frozen sections and places in formalin or distilled water and stains with Giemsa's stain (one drop of stain to 1 c.c. of distilled water). Great precaution must be observed as to cleanliness of vessels used. After one hour, places in a fresh staining solution and lets remain from twelve to twenty-four hours. When sufficiently stained, the section should be a deep-red to violet-blue. It is now placed in distilled water or in a concentrated solution of potassium alum until it becomes a bright blue. It requires but a short time in the potassium alum solution to differentiate the section. It should then be washed in water for a short period; too long washing is not good for the preparation. It is then mounted in glycerin jelly, cedar oil, or neutral Canada balsam.

Ravant and Ponselli (*loc. cit.*) take 30 c.c. of water and 30 drops of blood, added drop by drop. The hemoglobin becomes diffused through the water and after three hours a fibrinous clot forms. The

clot is withdrawn, washed several times to free it from water, cut into sections, and stained by Levaditi's method.

Levy-Bing (*Bulle. Méd.*, Paris, June 24, vol. xix, 49) stains with an alcoholic (methyl) solution of azure blue and counterstains with eosin. The *S. pallida* is stained an orange-rose color.

Reitman (*Deut. med. Woch.*, Leipsic and Berlin, June, vol. xxxi, 25, p. 997) first fixes the spread in absolute alcohol, washes in water, places in a bath of phosphomolybdic acid, again washes in water, stains with carbol fuchsin, washes in water, then 70 per cent. alcohol, and alternates with water until no more color comes away. The spirocheta is stained a deep red.

Follet (*C. R. Soc. Biol.*, April 20, 1907, t. lxii, p. 667) recommends collecting the saliva some time before a meal and staining with a mixture of glycerin, acid fuchsin, and carbolic acid, for the demonstration of spiral organisms. Another method, using a mixture of glycerin, methylene blue, and carbolic acid, he says has permitted him to observe from 200 to 300 spirals, "certainly not all the *S. pallida*." A third formula consisting of chloroform, methylene-blue, acid fuchsin, and carbolic acid, is more rapid and gives less precipitate and presents the classic coloration of the *S. pallida*.

Proca and Vasilescu (*C. R. de la Soc. de Biol.*, 1905, vol. lviii, p. 1044) recommend to fix the preparations in absolute alcohol for thirty minutes; then place in a bath composed of carbolic acid, 50 c.c.; tannin, 40 grams; water, 100 c.c.; fuchsin (2 to 5 per cent. alcoholic solution), 100 c.c., for ten minutes. Wash in water, and stain for ten minutes with carbol gentian violet.

Volpino (*R. Acad. di Med. di Torino*, July 14, 1905) allows sections to remain in a solution composed of nitrate of silver 0.5 grams, in 100 c.c. of distilled water for twenty-four to forty-eight hours. They are then washed in water and transferred to a solution of tannin, 3 grams; gallic acid, 5 grams; acetate of soda, 10 grams; distilled water, 350 c.c. Allow them to remain for fifteen minutes until they are brownish in color, wash in water, dehydrate, clean, and mount in balsam.

Bab (*Zeit. f. Geburtshilfe und Gyn.*, 1907, Band lx, Heft 2) thinks that the organism possesses an affinity for mercury equal to that for silver, and recommends trying mercury phenylate.

Noeggerath and Staehlin (*Munch. med. Woch.*, 1905, 31, 1481)

take 1 c.c. of blood from a vein, mix with 10 c.c. of 0.3 per cent. acetic acid in water, centrifugalize, and examine the deposit in the ordinary way.

McNeal (*Jour. Amer. Med. Assoc.*, February 16, 1907) recommends a mixture of methylene violet, methylene blue, yellowish eosin, and pure methyl alcohol. The stain is allowed to remain upon the smear for forty-five to sixty seconds, then immersed in about 10 c.c. of a 1 to 20,000 solution of sodium carbonate and the mixture stirred by tilting the dish. After one or two minutes' immersion the cover glass is removed, washed in distilled water, cleaned, dried upon blotting paper, mounted in water, and examined with $\frac{1}{12}$ inch objective.

Schereschewsky (*Deut. med. Woch.*, March 21, 1907, p. 462) fixes one minute in osmic acid vapor, passes the cover slip three times through the flame, then places it in a mixture of 1 part Giemsa's solution to 8 to 10 parts water. The preparation is heated in a Petri dish, upon a water bath, for ten to fourteen minutes, and when the cover slip presents a scum of a reddish color the procedure is at an end. It is said that by this method the preparations are so clear that the organisms can be observed with a dry objective.

Benda (*Berl. klin. Woch.*, April 15, 1907, p. 428 to 432; April 22, p. 480 to 484), by studying the *S. pallida* by the silver process of Levaditi, claims that the parasites are the same organisms that are found in spreads from organs, and not fragments of tissue elements.

Zabel (*Mediz. Klinik*, May 19, 1907, p. 580 to 582), in staining sections of organs, fixes in formalin and stains with nitrate of silver. The organisms appear larger than those made from fresh preparations stained with aniline dyes.

INOCULATION EXPERIMENTS UPON ANIMALS.—Metchnikoff (*Berl. klin. Woch.*, May 22, 1905) found the *S. pallida* in the glands and chancres of his inoculated apes (23 out of 31 experimental lesions), and Arnol and Salmon (*Ann. de l'Inst. Pasteur*, July 25, 1904) report upon the features of the lesions produced upon chimpanzees, a male and a female, both showing chancres which were identical, histologically, with those in man.

Piorkouski (*Berl. klin. Woch.*, December 19, 1904) injected 5 to 10 c.c. of blood from a patient under active mercurial treatment into

a horse, intravenously or subcutaneously. Four weeks later a maculopapular rash appeared, which, examined by various experts, was pronounced to be syphilitic in nature (no mention of *S. pallida*).

Neisser (*Deut. med. Woch.*, Berlin and Leipsic, xxxii, Nr. 13) has succeeded in inoculating monkeys with tertiary lesions, provided the lesion is not destroyed by suppuration or necrosis. He was also successful in producing positive inoculations, by using the nasal secretion, blood, and tissues from various organs, from children with inherited syphilis. He claims the bodies of children with inherited syphilis are swarming with parasites which can pass, by way of the blood, into the organs.

Hoffmann (*ibid.*) also reports experiments with monkeys, which have confirmed the contagious nature of the blood during the early stage of syphilis.

Metchnikoff and Roux (*Bull. de l'Acad. de Méd.*, May 18, 1906) report upon the inoculation of syphilitic material into apes and man, followed by the application of a strong mercurial ointment locally. They found that if this application was made within one to eighteen hours, it destroyed the syphilitic virus, but if made later than this, syphilis developed.

Neisser (*Bull. de la Soc. Francaise de Prophylaxie Sanitaire et Morale*, April and May, 1906, Nos. 4 and 5) proceeded upon these lines, but without success. On the contrary, though the mercurial ointment was applied one hour after the inoculation, the chancre developed in due course. He came to the following conclusions: That the chancre developed in the same manner in a mercurialized subject as in one not under that treatment, and that the disease became generalized in exactly the same manner among animals which had been mercurialized and those which had not.

Metchnikoff (*Brit. Med. Jour.*, October 19, 1907, p. 1075) at a later date experimented upon the prophylaxis of syphilis, using instead of mercury, atoxyl. This material was found to protect monkeys from the infection, even after a single dose. It was further found that the injection of atoxyl could be successfully carried out a week or even a fortnight after the inoculation. That the atoxyl had really neutralized the virus, was shown by the fact that the monkeys could be reinfected with syphilis some months after the atoxyl treatment.

He then spoke of giving atoxyl by the mouth, and concluded by saying that these preventive measures had been applied to man without any harmful after-effects.

Yancke (*Mediz. Klinik*, April 28, 1907, p. 486 to 487) took fragments of placenta, liver, kidney, spleen, and testicle from a syphilitic fetus of six months, macerated these in distilled water, and filtered under pressure through a Chamberlin filter. The filtrate was inoculated into the superciliary region of a monkey, and was followed by a slight infiltration, which reached its height in seven days. Forty-two days after inoculation he found a lesion similar to that noticed in primary syphilis. The long period of incubation was probably due to the small number of organisms contained in the filtrate. He attributed the result of this experiment to two factors: first, to the emulsion, and secondly, to the high pressure of $2\frac{1}{2}$ atmospheres.

Thibierge, Ravant, and Burnet (*La Semaine Méd.*, February 14, 1906, p. 80, No. 7) experimented upon macaque monkeys with fragments of enlarged gland, of papules, and chancres; inoculated them in a series of other animals, and found the *treponema pallidum* in the secretions and sections of tissue. They conclude that the experiment in passage, from the man to the animal, of the parasite speaks in favor of the organism being the pathogenic microbe.

Simonelli and Bandi (*Gazz. Degli Ospedali e delle Cliniche*, January 7, 1906) inoculated a female ape with material from a syphilitic perianal hypertrophic papule. In due time a chancre developed, but they were unable to demonstrate the *spirocheta pallida* in the lesions. They found certain masses of very delicate thread-like structures, some of which were straight, and others wavy.

Metchnikoff and Roux (*Ann. de l'Inst. Pasteur*, November, 1904, 761, and *ibid.*, *Bull. de l'Acad. de Med.*, 1905, p. 468) succeeded in infecting monkeys with syphilis, and in 4 out of 6 cases found *S. pallida* unaccompanied by other forms.

Hoffmann and Walter Bruning (*Deut. med. Woch.*, April 4, 1907, 553, 554) succeeded in inoculating rabbits and then inoculating the virus in monkeys. Two dogs were inoculated with the fragments of a chancre which was followed in sixteen and twenty-one days by keratitis of a specific character with the presence of *S. pallida* and clinically possessing the characters described in the monkey. By

scarifying the cornea they proved that laying bare of the bloodvessels is not absolutely necessary.

Bertarelli (*Central. f. Bakt.*, I. Origin., April 25, 1907, p. 790 to 793) has successfully inoculated a sheep and a dog with virus that passed seven times through rabbits by inoculations into the cornea. The cornea of the dog was scarified and smeared with the virus from the cornea of a rabbit, and sixteen days later a keratitis, specific in character, was noticed. In the dog the lesion was more extensive than in the sheep. The syphilitic nature of the lesions was confirmed by microscopic examination, and only two gave positive results, and the organisms were few in these two cases.

Hoffmann (*Berl. klin. Woch.*, March, 1905, xli) is said to have successfully inoculated four monkeys (2 *Macacus rhesus* and 2 *cerco-pithecus*) with syphilitic blood.

Finger and Laudsteiner (quoted by Flexner, *Med. News*, December 9, 1905) report a successful transmission of syphilis to the monkey by means of the inoculation of a large amount of gummatous material.

Kraus and Prantschoff (*loc. cit.*) inoculated monkeys—*Macacus rhesus*—with syphilitic material, and found that the initial lesion produced contained numerous *S. pallida*, being identical morphologically and tinctorially with the organism found in man. They were also successful in inoculating from one ape to another.

E. Finger and Laudsteiner (*Sitzungsb. der Kaiserl. Akad. der Wissensch. in Wien. Math. naturwiss. Klasse*, April, 1906, t. cxv, f. 3, p. 179 to 199) inoculated 6 monkeys, with the blood obtained from syphilitics in the full eruptive period, and not one inoculation was followed by a positive result. These data seem to show that the blood collects the cells in the course of the secondary eruption, and is not constantly virulent, and accords with the histological examination of the blood, which has proved that the *spirocheta pallida* is rarely found in this liquid. The milk of syphilitic women and the sperm did not contain the active virus as the experiments upon *M. cynomolgus* were entirely negative. They inoculated a "papion" with the centrifugalized sediment of the semen from a person with a chancre of the foreskin and lenticular papules of the arms and genital organs. Three weeks after inoculation the monkey showed an initial lesion. In one other experiment they used the semen of a person

presenting double interstitial orchitis (syphilis dating back three years), and the result was more marked than in the first. They also succeeded in engrafting syphilis upon inoculated monkeys, if they inoculated the virus five days after the appearance of the primary lesion. Later than this (five days) immunity begins to manifest itself in the animal, and it is impossible to obtain positive results. They also found that there is an absence of absolute immunity of the skin in the course of secondary and tertiary lesions. They then inoculated large quantities of the virus in subcutaneous pockets, and they have seen not only in the course of the eruption, but also during the tertiary stage, local lesions that were syphilitic in nature. They made control experiments with heated virus with negative results.

These experiments confirm the work of Neisser (recent meeting of Congress of Dermatology, held at Berne), and also the observations of Metchnikoff and Roux, of Finger and Laudsteiner, of Kraus, Neisser's collaborators, Halberstadter and Baerman, and Siebert. Among these last the most important have certainly been those who have proved that the active virus is in the tissues, as the marrow, spleen, lymphatic nodes, and testicles of the inoculated monkeys. Neisser found that eight hours after the inoculation, if the scarified area was extirpated, it hindered only the development of primary syphilis, as in this short space of time the microbe of syphilis had invaded the surrounding tissues, and infiltrated very rapidly all the organs. He also found that previous to the development of the chancre the bone marrow and spleen contain the active virus of syphilis and is inoculable into sensitive monkeys. This seems to prove the inefficiency of extirpation of the primary lesion.

Salmon (*C. R. Soc. Biol.*, February 16, 1907, t. lxii, p. 254) remarks upon the work of Finger and Laudsteiner upon the reinoculation of tertiary syphilitics being followed by the formation of lesions, reproducing the aspect of tuberculous and ulcerating syphilides. He has obtained positive results in only 2 out of 14 instances, and these were papulosquamous eruptions of contestable nature. He concludes that "the immunity co-exists with persistent infection indefinitely; and that the syphilitic possesses an absolute cutaneous immunity against reinfection from the outside."

Siegel (*Central. f. Bakt.*, I. Origin., March 5, 1907, t. liii; also March 21, 1907) claims to be the first to show that syphilis could be inoculated into the rabbit; he was the first to show that the organs of inoculated monkeys contained the active virus; he claims priority for the inoculation of the virus under the skin, and that he was able to obtain with great frequency the cutaneous manifestations in the chimpanzee, and that the examination of the internal organs, and especially the liver, should be carried out to consider the infection of a specific nature.

Hans Bab (*Zeit. f. Geburtshulfe und Gyn.*, 1907, Band lx, Heft 2) did not succeed in infecting apes by inoculating them intravenously, intraperitoneally, or subcutaneously, and expresses himself as being surprised that the intravenous inoculations were negative, as congenital syphilis offers itself as an exquisite example of a blood infection.

Rubbing the virus into the femoral vein and into incisions into the lymph glands also proved without result. In two instances inoculation into the parenchyma of the scrotum of the ape was followed by syphilis. The first animal became resistant toward a second attack. The second ape was killed after thirty-six days, and with the marrow two other monkeys were successfully infected. Those animals inoculated subcutaneously showed without doubt toxic symptoms, emaciation, cachexia, and high mortality.

The spirochetæ are by no means always present in the liver and spleen swelling, and this condition is perhaps caused by its toxin. He claims that the penetration of the virus into the abdominal cavity may occur through the tubes, and that it is also possible that the virus may be mixed with the semen in the prostate or urethra.

CULTIVATION.—Leuriaux and Gelts (*Cent. f. Bakt. und Parasit.*, Orig., 1906, Band xli, p. 684), in 42 lumbar punctures, obtained growths of *S. pallida* in 3 instances. One part neutral bouillon was added to 2 parts spinal fluid, and the mixture placed at 37° C. for several days. Centrifugalized twenty minutes and then the sediment spread over coagulated pork serum. An ivory-white moist film was the result. Smears from young cultures showed bodies like cytorhyctes luis, then a trypanosome, then a spirocheta.

De Souza, Jr., and Pereira (*Berl. klin. Woch.*, 1905, 44) tried culti-

vating the organism in 5 per cent. each of sodium citrate and sodium chloride, but with negative results.

Bertarelli and Volpino (*loc. cit.*) failed in all efforts to cultivate the organisms, as did Muhleus (*loc. cit.*).

REMARKS.—From the *resume* of the literature herein recorded, although by no means complete, it seems that the majority of observers look upon the *S. pallida* as the probable cause of syphilis. I have been able to collect references of 1210 lesions, including congenital syphilis, and in this number the parasite was present in 958. To enumerate still further, it may be mentioned that of 333 chancres examined, positive results were obtained in 299, and negative findings in 34. Of 43 papular eruptions, 35 were positive and 8 negative; of 33 mucous patches, 23 were positive and 10 negative; while of 122 roseola, 87 proved positive and 35 negative.

In the lymph nodes, or more properly, the juice of the nodes, only 6 positive findings are recorded of 24 examined. Of 35 condylomas, 22 were positive and 13 negative. Of 435 cases simply described as syphilitic, not definitely setting forth the lesions, 361 were positive. Of gummata there were records of only 11 being studied, 2 of which were positive and 9 negative; in 47 cases of tertiary syphilis examined, negative findings were recorded in all. By far the highest percentage of positive findings were in cases of congenital syphilis, for out of 127 instances of this form of the malady, in 123 the parasite was found.

My own personal observations have convinced me that the *S. pallida* is the probable cause of syphilis. In congenital syphilis, although the organism has been found most constantly, it is not always generally distributed in all the organs and tissues. It may only be demonstrable in one or two organs, as the spleen and liver, or in the kidney, or skin, and then only in certain areas of these structures.

Anyone who has had histological training should not mistake the parasite, when stained by the Levaditi method, for any tissue fibers in the different organs, while in spreads from lesions or organs it most certainly takes a practised eye to differentiate between delicate shreds of tissue and spirocheta, especially when stained by the aniline dyes or any of the stains for blood.

Another point must also be borne in mind, and this is, the certain disappearance or diminution in the number of parasites when

local or general treatment is resorted to. The examination should be made as early as possible, and I believe that the best stains to use for spreads from lesions or organs are Giemsa's azur-eosin and the blood stain of either Wright or Leishman. *December 12, 1907.*

The Coördination of Gastric and Intestinal Digestion through the Action of the Pyloric Sphincter.

By W. B. CANNON, M.D.¹

ABSTRACT.

(From the Laboratory of Physiology in the Harvard Medical School.)

(OBSERVATIONS on the passage of food from the stomach, made by means of the x-rays, show that the stomach is emptied *progressively* during the course of gastric digestion, by occasional discharges through the pylorus.

Mechanical agencies, either in the stomach or in the intestine, play an unimportant part in controlling gastric evacuation; for (1) the occasional discharges through the pylorus are not the result of momentarily deepened peristalsis, and (2) the upper intestine in normal conditions is not sufficiently filled or distended to check the outgo from the stomach.

Observations on chemical conditions in the stomach have hitherto been defective for judging the mechanism of the pylorus, because the food given at different times has not been identical in amount nor uniform in consistency, and the difference in the chemical reaction of the two ends of the stomach has not been distinguished. Furthermore, these studies, like the observations of Hirsch, Serdjukow, and Tobler, that acid in the duodenum checks gastric discharge, have failed to distinguish between two factors always concerned in the passage of food through the pylorus.

The two factors are (1) the pressure at the pylorus due to recurrent peristalsis, and (2) the action of the pyloric sphincter. The x-ray method shows that during gastric digestion peristaltic waves are

¹ Read by invitation.

passing, not occasionally, but continuously. Since the discharge from the stomach is not continuous, but occasional, the control must rest with the pyloric sphincter.

It is necessary to explain the intermittent closure of the pylorus, the usual closure, and the occasional opening. It is also necessary to explain why, for example, carbohydrates begin to leave the stomach early and depart rapidly; whereas proteids of the same amount and consistency begin to leave the stomach only after some time, and then depart slowly.

These facts can be explained on the theory that acid in the antrum opens the pylorus, acid in the duodenum closes it. Because the acid in the duodenum is soon neutralized, the closure of the pylorus is intermittent.

That acid in the antrum signals the opening of the pylorus is indicated by the following evidence: (1) moistening carbohydrates with NaHCO_3 retards their normally rapid exit from the stomach; (2) feeding proteids as acid proteids remarkably hastens their normally slow exit; (3) observations through a fistula in the antrum show that an acid reaction closely precedes the initial passage of food through the pylorus, that the introduction of acid causes pyloric opening, and that delaying the acid reaction causes retention of the food in the stomach in spite of strong peristalsis; (4) when the stomach is excised and kept alive in oxygenated Ringer's solution, the pylorus is opened by acid on the gastric side.

That acid in the duodenum keeps the pylorus closed is shown by the following evidence: (1) acid in the duodenum inhibits gastric discharge (observations of Hirsch, Serdjukow, and Tobler), and as shown above, the effect is not due to stoppage of peristalsis, but to closure of the pylorus; (2) the stomach empties more slowly than normally when the tying of pancreatic and bile ducts prevents alkaline fluids from neutralizing the acid chyme in the duodenum; (3) the discharge of proteid becomes rapid if the pylorus is sutured to the intestine below the duodenum, or if a ring is cut through the muscular coats immediately beyond the pylorus. The effect from the duodenum is thus a local reflex mediated, like movements of the small intestine, by Auerbach's plexus.

Evidence for the acid control of the pylorus is also found in the

application of the theory to previous observations on gastric discharge. Proteids leave the stomach only after considerable delay, and then emerge slowly; this fact can be explained (1) by the slow development of a marked acid reaction in the stomach due to the preliminary union of acid with proteid, and (2) by the large amount of acid borne into the duodenum by proteid chyme. Carbohydrates leave the stomach early and rapidly—a result to be expected, since the acid secreted upon them does not unite with them, and is at once present to open the pylorus. The peculiar rates of discharge of combinations of these food-stuffs are also readily explained on the theory above stated. The fitness of the theory to explain established facts gives it additional support.

The rapid exit of water through the pylorus without change of reaction, and the similar rapid exit of raw egg-white—facts not in accord with the acid control—are accounted for on the assumption that conditions not favoring gastric secretion are attended by a low pyloric tonus, and *vice versa*. Reasons are given for this assumption. The rapid exit of coagulated egg-white, exceptional among proteids, is explained by its slow union with the secreted acid. Fats leave the stomach very slowly. Like water and raw egg-white, they do not stimulate gastric secretion; but they may become acid in the stomach by the separation of fatty acid. Their very slow exit can probably be accounted for largely by the fact that when fats are fed, the pancreatic juice, instead of decreasing, increases the acidity of the duodenal contents.

Strong support for the acid control is found in its relation to other processes in the stomach and duodenum. The retention of food in the stomach until the antrum contents are acid is necessary (1) for the proper continuance of gastric secretion and (2) for the accomplishment of gastric digestion. Such retention is also necessary in order (3) that the chyme emerging into the duodenum may bear with it the acid required to cause the flow of pancreatic juice and bile, and (4) that the pylorus may be held closed until these important secretions are thoroughly mixed with the acid chyme.

The facts presented bring the pyloric mechanism under the “law of the intestine,”—the acid when above (in the antrum) causes a

relaxation of the sphincter which is below, and the acid when below (in the duodenum) causes a contraction of the sphincter which is above.

DISCUSSION.

DR. J. DUTTON STEELE stated that in the estimation of the symptoms of so-called gastric atony no account was usually taken of the intricate mechanism described by Dr. Cannon, and that too much importance had been placed on slight variations of gastric secretion. A method was needed to estimate clinically the action of the pylorus in various gastric diseases.

DR. BRUBAKER stated that one fact which had come to his notice was apparently unexplained by the description of Dr. Cannon, and that was the firm persistent contraction of the pylorus after death. The pylorus, according to Dr. Cannon, was a passive structure with a certain amount of tone. Attempts, however, to relax the pylorus after death by washing out the stomach and duodenum always failed.

DR. CANNON, in answer to Dr. Brubaker, said that an answer could be given to his question by a simple experiment. A section of the alimentary canal is placed in Ringer's solution through which oxygen is allowed to flow. By attaching a lever to the muscle and cutting off the oxygen a contraction of the muscle would be recorded due to the absence of oxygen. The pylorus in cadavers, therefore, was contracted because of the absence of oxygen.

January 9, 1908.

Interpretation of the Appearances Seen in a Peripheral Nerve.

BY H. H. DONALDSON, M.D.¹

ABSTRACT.

UTILIZING the characters of the medullated fibers as seen in the cross-section of a peripheral nerve, attention was called to the fact that the muscular branches always contain large numbers of sensory

¹ Read by invitation.

fibers, and only the smaller cutaneous branches are composed of fibers of one class. Both sensory and motor fibers are alike in the fact that in osmic acid preparations of fresh and normally extended nerves the area of the axis cylinder is equal to the area of the surrounding medullary sheath. The foregoing method has not yet been employed for the study of pathological variations in this relation.

In their course, fibers divide so that in general the number of fibers distal to a given point is greater than the number of fibers on the proximal side of it. It follows from this that a proximal lesion will cause the destruction of a larger number of fibers the farther distal to the lesion the sections are taken. Both sensory and motor fibers undergo this sort of division, and there are a number of interesting physiological consequences which must be connected with this phenomenon in the case of the sensory fibers.

The section of any peripheral nerve shows great differences in the diameters of the fibers in it. These differences can be interpreted as follows:

The fibers of larger diameter run the shorter course, and will be distributed to more proximal segments of the limb than those of smaller diameter with which they are associated. In general, the fibers of larger diameter are older than those of smaller diameter, and might therefore be expected to react differently to pathological influences. The fibers of larger diameter also are outgrowths of cell bodies of large diameter, both in the spinal cord and in the spinal ganglion, and their physiological activity is modified by this fact.

DISCUSSION.

DR. D. J. MCCARTHY: The interesting facts presented in Dr. Donaldson's paper might be applied to the pathological processes in the nervous system. The facts presented in connection with the relative area of the axis cylinder and of the sheath of Schwann, have already been applied, in a rough way, to neuropathology. We are accustomed, after studying large numbers of sections of peripheral nerves, to assign relative areas to the axis-cylinder and the sheath of Schwann. When the area of the axis cylinder is diminished, we are

accustomed to speak of atrophy of the axis cylinder, and on the other hand, when it is increased, to speak of a swollen condition of the axis cylinder. In the latter group of cases a careful study would reveal a distinction between the relative and actual swollen condition of the axis cylinder. In simple atrophic processes, such as are met with in chronic wasting diseases and in which there are no active degenerative processes, the relative loss of the sheath of Schwann may possibly be shown.

If we assume the correctness of the neuron theory, the division of the sensory nerve fibers is not at all surprising. That portion of the sensory nerve fibers in the peripheral nerves distal to the spinal ganglia may be considered as a dendrite, and the branching of dendrites is the rule rather than the exception. In relation to the relative size of the nerve fibers in the proximal and distal portions of a nerve, the possibility should be taken into consideration of a so-called chronic diminution in the cross areas of the nerve from the proximal to its distal distribution.

DR. DONALDSON: I am indebted to Dr. McCarthy for pointing out the pathological applications of the observations just presented. Concerning the last point made by Dr. McCarthy, namely, the possible decrease in the diameter of nerve fibers during their course from the centre to the periphery, I would merely call attention to the fact that the current views are based on the observations of Schwalbe, 1882. These observations have little value, and a careful testing of the point on several occasions in my laboratory has convinced me that such conical diminution of the fiber does not occur to an appreciable degree. The absence of fibers of large diameter from the more distal portions of the spinal nerves depends upon the fact that they have been distributed already by way of more proximal branches.

January 23, 1908.

The Value of Stained Smears in the Diagnosis of Diphtheria.

By E. BURVILL-HOLMES, M.D.

I THINK that it is undoubtedly the opinion of men who are conversant with diphtheria, both from its clinical and bacteriological aspects, that in no case is the specific organism absent from the parts involved. True, there are a few men—fortunately very few—who still have a tendency to deride or consider of little moment the findings of the bacteriologist, and who insist that mere inspection of the throat, etc., is sufficient for diagnosis, the aid of the microscope not being essential. These men either cannot or will not accept what are facts, not theories merely, and that is, that organisms other than the diphtheria bacillus can produce pseudomembranes identical with those produced by this latter organism, and I think mainly because they know little about, and have worked less in, bacteriology. That this has been and can be proved beyond the peradventure of a doubt must be apparent to anyone who has kept abreast of the literature or who has made investigations for himself. Klebs¹ himself observed a whole family epidemic of false diphtheria, which was caused by a large monococcus of the group monades. Ballouche² has shown that typical diphtheritic pseudomembrane can be produced not only by the streptococcus, but by the staphylococcus and the pneumococcus. Moreover, according to Bourges and Futterer, paralysis identical with that which often follows in the wake of a diphtheria intoxication has been seen as a sequel of a pure streptococcic angina. Again, Fränkel's pneumobacillus can be responsible, and at times is, for errors in diagnosis. The symbiosis of Vincent's spirilla with the fusiform bacillus gives rise to an exudate, a pseudomembrane, call it what you will, which on more than one occasion, to my personal knowledge, has caused an individual unfortunately so afflicted to be sentenced to an enforced sojourn in a ward of the diphtheria pavilion. Another organism, which of late years we have been led strongly to believe is the etiological factor of syphilis—the *Tryponema pallida*—has been, while not to the same extent, guilty of the same thing. To quote from a report of the local Govern-

ment Board of London,³ "It must be remembered that membranes produced by bacteria other than the Klebs-Loeffler bacillus may appear in the throat, and that in many cases the clinical phenomena prove to be of little assistance; in these cases a careful bacteriological examination should always be made." Filatov⁴ says: "In the diagnosis of the throat, clinicians are guided not by the anatomical changes of the mucous membrane, but by etiological factors, namely: Diphtheria of the throat is an inflammation of its mucous membrane produced by the Klebs-Loeffler bacillus. It is immaterial whether the throat be affected by a croupous exudation or a catarrhal one; as soon as we find that in a given case the cause of the sore throat is Loeffler's bacillus, we should regard such morbid process as diphtheritic, and should so characterize it. Since not only Loeffler's bacillus is liable to produce diphtheritic inflammations of the mucous membranes, *i. e.*, membranous exudations, but other microbes may also have similar action, it is obvious that the presence of a membranous coating alone on some of the mucous membranes does not prove that we have to deal in any given case with a case of diphtheria." Personally I had the privilege and opportunity while at the Municipal Hospital of observing, both clinically and bacteriologically, many cases in which inspection of the throat alone was responsible for errors in diagnosis, and I am certainly convinced that without a bacteriological examination one is not justified in saying that this case is one of diphtheria and that one is not. Let me cite two cases as examples:

CASE I.—Miss C., a trained nurse, while caring for one of her associates who had been sent to the hospital suffering from scarlet fever, was taken ill with headache, temperature, and angina. When her throat was first examined some slight whitish pseudomembrane was noticed on both tonsils, which later spread. It was considered a case of diphtheria, and particularly in view of a report by one of the resident physicians—who had in my absence examined a smear—that the Klebs-Loeffler bacillus was present in very large numbers. The patient was transferred to the diphtheria pavilion, and antitoxin was administered. On my return to the hospital I was requested to examine the smear, and though it was carefully gone over, not a single diphtheroid organism could be found, the

organism mistaken by the first examiner being the *S. lanceolatus*. A culture made from the same swab substantiated this, the latter organism being in pure culture. Although repeated cultures were made, the Klebs-Loeffler bacillus was never found. Twenty-four hours afterward, or thereabouts, the patient developed a cough, with some dyspnea, and while physical examination of her chest revealed little or nothing, her subjective symptoms and her subsequent temperature reading made a diagnosis of central pneumonia more than probable. I may add that two days after the administration of the antitoxin she developed violent pains, with swelling of her joints, and later a marked pericarditis with effusion. Whether the pneumococcus was responsible for the entire train of troubles, I am not prepared to say. I believe that it was. A blood culture remained sterile.

Here then is a case where the pseudomembrane was undoubtedly pneumococcic in origin. It certainly was not diphtheritic, since, as I said, after repeated cultures the specific organism of that disease was never found.

CASE II.—W. J., a young male, was admitted to the diphtheria wards, suffering from malaise and angina. Examination of the throat showed a dirty white membrane on both tonsils and to a slight extent also on the uvula. Diagnosis other than diphtheria was not entertained at first, and he was, and correctly, so treated. However, repeated negative cultures with persistence of the membrane prompted the physician in charge to look further into the cause of the trouble, and accordingly he was sent to the laboratory with a request that a smear be made and examined for the *T. pallida*. This was done, that organism found, appropriate treatment was adopted, and the exudate promptly disappeared.

And so case after case might be recorded if time and space permitted. To repeat, then, I say that every case of diphtheria, providing the throat be properly swabbed and cultured, the culture incubated at the proper temperature—an important factor—and examined by a competent bacteriologist, will invariably demonstrate the bacillus. Conversely, and I cannot do better than to quote Professors Kathnack and Hardy,⁵ “from the investigations made all over the world, we must refuse to call any lesion diphtheria unless it is associated with

the bacillus. The bacillus asserts itself with an authority which must put aside any preconceived notions."

There are those, of course, who will argue that the bacteriological investigation in a certain case is not necessary, because if so suspicious as to be differentiated with difficulty, it should be for the welfare of the patient, as also those with whom he might come into contact, that the treatment and precautions against contagion should be in accord with the assumption. Conceded: until the diagnosis can be clinched as it were by the microscopist, all this should be done, and promptly. If, however, the subsequent treatment is to be carried along logical and scientific lines; if that chaos in households which a wholesale disinfection entails is to be eliminated; if the perturbed mind of a parent consequent upon the knowledge that her child has diphtheria is to be set at rest, and lastly, if statistical records are to be open to less criticism than they unfortunately are at the present time, then it is essential that bacteriological studies should be made in every case of exudative angina. Again, the argument has been advanced that since diphtheria organisms are found in healthy throats, a case in accordance with smear and cultural findings may be deemed one of diphtheria, when, as a matter of fact, that organism may be taking little or no part in the diseased process. This leads us to the question as to how many well persons harbor the organisms in their throats. According to a report of a Committee of the Association of the Massachusetts Board of Health⁶ persons not exposed to the contagion revealed the germ on culture in 3 per cent. of cases. In the eastern part of the United States the percentage was lower, or 1.39 per cent. On the other hand, their report states that in the exposed the percentage rose to from 8 to 50 per cent. If this latter is correct, then my experience must be somewhat unique. Smears and cultures from the resident physicians and nurses on duty in the diphtheria wards were taken at various periods, and of the eighty or ninety so studied, only two gave positive results, or 2.2 per cent. Both were nurses, newcomers, having been in the hospital but four days, one of whom went down with the disease the day following the issuance of the report. To obviate any prejudice in the matter, the cultures were made by one of the resident physicians and sent to

the laboratory with those from the patients, and under fictitious names. At any rate, the number of well persons whose throats contain diphtheria organisms are so comparatively few that when found in diseased throats we are justified in the inference that the case is one of diphtheria.

THE VALUE OF SMEARS. Early diagnosis of pseudomembranous anginas is imperative, first, so that prompt and proper remedial measures can be instituted, and secondly, if contagious, immediate isolation or removal of the patient to the hospital be effected. That diphtheritic anginas frequently present the typical text-book pictures of follicular tonsillitis is a recognized clinical experience with many practitioners. Indeed, more than one life has been sacrificed because the clinician failed to appreciate this fact. It is not so many years ago that in an Orphan Asylum on the outskirts of the city, such an error cost the loss of five lives and the expenditure of about four thousand dollars. In the following cases the Klebs-Loeffler bacillus was undoubtedly responsible for the trouble, though the clinical picture was that of follicular tonsillitis:

CASE I.—E. L., a girl, aged ten years, was sent to the hospital from her suburban home, with a request that she be admitted, as she was supposed to be suffering from diphtheria. On admission, inspection of her throat revealed two much enlarged and congested tonsils, each having thereon a small circumscribed dot of yellowish pultaceous exudate. Her condition was considered to be that of tonsillitis, and not diphtheria, and the appearance certainly would justify such a diagnosis. However, before discharging her to her home a smear was made from the exudate. This showed an exceedingly large number of diphtheria organisms, and cultures made from the same swabbing showed on the following day a pure culture of the bacilli. Furthermore, a guinea-pig test proved the germs to be virulent.

CASE II.—F. K., a druggist, presented all the clinical symptoms of a follicular tonsillitis—prostration, high temperature, and considerable angina. The tonsils were very large, reddened, and small dots of yellowish soft exudate were to be seen filling the crypts. It was considered by the attending physician, a laryngologist, as a case

of tonsillitis, though, to give the patient the benefit of any doubt, antitoxin was administered. A smear and culture were made, and both showed the bacillus almost exclusively. These organisms were also virulent.

CASE III.—B. A., a boy, aged seven years, presented all the symptoms of a follicular tonsillitis, and it was so diagnosticated. Twelve hours later the small yellow dots of exudate had coalesced, but there was nothing in the appearance of the parts to prompt the attending physician to alter his initial opinion. Only when it was too late did he realize what he had to deal with, and the child died.

The following case was one in which a diagnosis of diphtheria was made, but which smear and culture proved undoubtedly to be staphylococcic infection:

CASE IV.—Miss H., a trained nurse, was sent from one of the hospitals, where she was on duty, with a diagnosis of diphtheria. On one tonsil was a small white patch of exudate about 5 mm. in diameter. The tonsils were not markedly congested or enlarged; there was slight dysphagia. A smear was made, but no diphtheria organisms were noted, even after a long, careful search. She was immediately discharged and the next day without any treatment whatsoever she was entirely well, at least to all intents and purposes. The culture made from the same swab from which the smear was made showed the following day a pure culture of the *Staphylococcus pyogenes aureus*.

We see that in Cases I, II, and IV smears served a good purpose. Had inspection alone been relied on, it is easy to see how disastrous might have been the results and particularly as regards Case I, where the child would have been sent home, there to mingle with other children. To have awaited the results of culture would have meant detention in the hospital wards for at least twelve hours. Removal of Case II to the Municipal Hospital was consummated one hour after the visit of the physician, in lieu of being deferred for twenty-four hours had culture alone been relied on, thus obviating the dangers of contagion, as the patient was a resident in an apartment house. The smear saved Case IV from being subjected to antitoxin treatment and forced detention in the diphtheria ward unnecessarily. Had a

smear or culture been made in the case of the small boy, a life might have been saved.

Being desirous of ascertaining the value of smears as compared to cultures, I made and examined a considerable number from the noses and throats of patients admitted to the diphtheria and scarlet fever wards. My conclusion is that they are of great value, indeed in experienced hands as valuable as cultures, because even the latter, owing to careless culturing for the most part, do not always reveal the true state of affairs. Of special value are they when an immediate diagnosis is required. The experience of Welch and Schamberg,⁷ who examined a large number of smears also at the same hospital, is much in accord with my own. It would be a mistake for me to leave the impression that it is easy to positively identify the organism in smears without some experience, as is demonstrated by the smaller percentage of errors as this experience is augmented. Smears obtained from the nose in particular are oftentimes difficult to interpret. But then I do not believe that either smears or cultures are of much value in diagnosing nasal diphtheria without the addition of clinical evidence or guinea-pig tests, because the nose is a frequent habitat of an organism, the morphology and cultural characteristics—to the exclusion perhaps that it renders bouillon very slightly turbid—are identical with those of the Klebs-Loeffler bacillus. They do, however, lack virulence. From the nose too, even in culture, I have observed that virulent organisms only rarely show polar staining or granules, as is so frequently seen in those from the throat. As a matter of fact, polar staining and granules are often absent in organisms from throats as examined in the smear. The grouping of the bacilli rather than their actual morphology I consider the more valuable in recognizing their presence. The bacilli will be found in most part lying together in groups of two or more, paralleling each other or radiating from each other like the spokes of a wheel. This is most characteristic both in smears and cultures.

Of 529 smears examined, only in 31 did they fail to correspond with the cultures, and in every instance the smear was made from the same swabbing as the culture. This is a difference of 5.8 per cent. In 12 of these cases the report on the smear was positive when the

culture showed no organisms, although the case was one of diphtheria, as subsequent cultures proved. In 3 instances I can account for this, as, owing to a gas regulator which refused to regulate and being a warm night in summer, the temperature of the incubator rose to 52° , a degree of heat we know to be prejudicial to the growth of the Klebs-Loeffler bacillus. In the remaining cases the discrepancy was due to the fact that other organisms doubtless predominated and choked out the diphtheria bacillus. Whatever the reason, it showed that here, at least, the smears were of more value than the cultures. This, then, leaves 24 cases in which the error was on the wrong side, or 4.1 per cent.; 6 of these, again, were from the nose, a site from which, as I remarked before, smears were at times difficult to interpret. In 2 of these 6 cases, long search of the smear failed to show a single organism of any kind, and quite recently I was asked to examine a nose smear from a young physician, which showed, and then only after hunting for some time, a single pair of organisms lying side by side, on which he was isolated and later sent to the Municipal Hospital. The culture showed the bacillus almost solely. Withal, then, with these results, because even cultures depending upon personal equation, incubator troubles, or choking out of the diphtheria bacilli by more rapidly growing germs will show a degree of error to the extent of at least 4 per cent., I do not think it without the pale of consistency to claim a value for smears almost as great, if not as great, as cultures. I should suggest that cultures as controls ought always to be made, and it would be time well spent if every young clinician would make himself conversant with this means of diagnosis.

CONCLUSIONS.—No pseudomembranous angina should be considered as one of diphtheria if after careful and proper culturing and competent examination the Klebs-Loeffler bacillus is absent.

Cultures or smears should be made in every case irrespective of the appearance of the parts involved.

Typical pictures of follicular tonsillitis frequently show large numbers of virulent bacilli.

When in doubt, and until the smear or culture can be examined, all cases should be treated as diphtheritic in origin.

Without clinical evidences or guinea-pig tests, smears and cultures from noses are not of much value.

The grouping of the bacilli rather than their morphology is the more valuable aid in the identification of the bacillus in both smear and culture.

For immediate diagnosis smears are of great value; indeed, from the results obtained in the cases mentioned it would tend to show that they were as valuable as cultures.

DISCUSSION.

DR. R. C. ROSENBERGER stated that he had always been wary of making a diagnosis from the morphological and tinctorial characteristics of diphtheria bacilli alone unless the bacilli were present in great numbers in the smear. He always insisted on cultures. In his own experience he had found Loeffler's methylene-blue stain the most satisfactory. He had noted marked variations in the morphology of the organism at different stages of its growth. His test for virulency was the guinea-pig inoculation.

DR. GEORGE FETTEROLF referred to the practical application of the smear method of diagnosis. In two cases of his, reported by Dr. Holmes, a positive diagnosis had been made, antitoxin administered, and removal to the hospital consummated an hour after the case was first seen. In both cases the diagnosis could not have been made from the throat symptoms alone. In another case, not reported by Dr. Holmes, which had symptoms very suggestive of diphtheria, smears showed the infection to be due to staphylococcus, and the patient was saved the annoyance of an injection of antitoxin.

DR. HOLMES, in closing, said that he also had found Loeffler's methylene-blue the most satisfactory stain. In his diagnosis he did not rely so much on the morphology of the organism as on its arrangement. The organisms which might be mistaken for diphtheria bacilli are usually found singly and not side by side. Cultures are of value in confirming the diagnosis made from the smear.

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Transplantation of the Parathyroid Glands.

By W. S. HALSTED, M.D.,¹

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I FEEL that I may be appearing this evening under colors not altogether true. Accepting the flattering invitation of the Philadelphia Pathological Society and of the Academy of Surgery of Philadelphia, I had expected ere this to be able to announce facts more definite and of greater value as the result of our work for the past year and a half on the transplantation in dogs of the parathyroids. But lack of time and many vicissitudes in connection with the laboratory work have made impossible the entire fulfilment of my plans.²

Begun in the winter of 1906-1907 and continued with interruptions until the present time, the work was undertaken to determine the course to be pursued by the surgeon when a parathyroid gland has been accidentally removed or deprived of its blood supply, and in the

¹ Read by invitation.

² Our experiments have been continued and results obtained which enable me now to make more definite pronouncement on the subjects, both of the auto- and iso-transplantations, than was possible at the time of the joint meeting in Philadelphia of the aforementioned Societies.

hope that it might be attended with such success as to justify the attempt to transplant this glandule from man to man. In the course of the work many questions have arisen, and we find ourselves still on the threshold of the investigation. A patient suffering greatly from subtetanic hypoparathyroidism as the result of two operations by myself upon a large colloid, suffocation-producing goitre anxiously awaits the results of these experiments. For three years tetany has been averted and the status parathyroprivus made endurable by hypodermic injections of the parathyroid nucleoproteids (Beebe) and for a few days¹ by the administration of calcium lactate (MacCallum and Voegtlin²).

My first transplantations of the parathyroid glands,³ and the first of which I know, were made into the thyroid gland of the donor; next into the spleen, on the announcement by Payr of the successful transplantations of the thyroid gland into this organ; and finally (1907-1908), on the appearance of Leischner's⁴ contribution (von Eiselsberg's clinic), within the sheath of and behind the musculus rectus abdominis, and in the midline, preperitoneally.

Leischner succeeded in a small percentage of his cases in transplanting preperitoneally the parathyroids in rats. These were autotransplantations, the donor being the donee. Pfeiffer, Hermann, and Mayer made⁵ two successful autotransplantations in puppies.

Biedl, commenting on the unsuccessful attempts of Foges, Kreidl, and himself to transplant ovaries, testicles, and suprarenal glands, contrasts these failures with his success in transplantation of the parathyroid glands. He states that a year prior to his report,⁶ he transplanted in two dogs into the spleen "foreign" parathyroids, and

¹ May 10. This patient has been taking calcium lactate uninterruptedly for two months, and with excellent results.

² Reference has been made to this case in previous publications. In the two operations only one parathyroid gland was removed (specimens examined by Dr. MacCallum and myself), and this was recognized the instant the final resection-cut into the right thyroid lobe was made. It was not until six months later, in the autumn of 1906, that it occurred to me to attempt the transplantation of the parathyroids.

³ Am. Jour. Med Sci., 1907, vol. cxxxiv, No. 1.

⁴ Leischner. Archiv für klinische Chirurgie, 1907, Band lxxxiv, Heft 1, p. 208.

⁵ Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1907, Band xviii, Heft 3, p. 377.

⁶ Wien. klin. Woch., February 27, 1908, p. 304.

"after a time" removed both thyroid lobes as well as the parathyroids. One animal lived seven months without a trace of tetany and finally died of what seemed to be "cachexia thyreopriva." The spleen contained, the report states, two well healed, intact parathyroid glands. The second dog had tetany of short duration. It recovered, however, entirely, still lives, and consequently has, the author believes, parathyroids in the spleen which are functionally sufficient.

With the exception of the two cases of Biedl, I find no report of the successful isotransplantation of the parathyroid glands, and besides my own the only successful autotransplantations of these glandules in dogs are, perhaps, the two reported by Pfeiffer, Hermann, and Mayer. As to the successful isotransplantations of Professor Biedl, I confess to a little surprise in view of the facts that no deficiency was created before the transplantation and that both parathyroid glands survived in both animals. The absolute, the functional proof is lacking in these cases, inasmuch as the transplanted glands were not excised during life.

Sixty dogs, approximately, have been used in this investigation and more than 120 parathyroid glands transplanted.¹ The technique has been precisely that of the operating rooms of the Johns Hopkins Hospital.

RESULTS (*winter of 1906-1907*). *Autotransplantation.* Parathyroid deficiency is of necessity created in the autotransplantations. Of five autografts into the thyroid lobes of three dogs, three were successful (macroscopic and microscopic proof). Of eight autografts into the spleens of three dogs, one only succeeded (macroscopic proof). In no instance was functional proof of the success of these transplantations obtained. Such proof cannot, of course, be so convincingly obtained in the cases of implantation to the thyroid because of the lack of certainty that no parathyroid tissue except that transplanted remains

¹ In 1906-1907, I was assisted by members of my house staff and by medical students, especially Messrs. Chestnutt and Dinsmore; this year Dr. Hennington has rendered me great service in the operative work, and Dr. Cushing has shared with me his operating room in the Hunterian Laboratory. The vicissitudes have been many—pneumonia, distemper, accidents, and escapes. On one occasion the laboratory was broken into, presumably by a discharged employee, and many of my most prized dogs set at large. Some of these dogs were awaiting the ultimate functional test of the transplantation after two or more operations.

at the time of the final operation, at which well nourished thyroid tissue, sufficient to insure the life of the transplanted parathyroid gland, must be left.

Isotransplantation. In five cases (dogs K, L, M, N, and O), 2, 7, 5, 5, and 8 parathyroids, respectively, were transplanted into the spleen. In only one dog (K) was a parathyroid deficiency created. In no instance was the transplantation successful; furthermore, tetany supervened and death occurred just as promptly, after removal of the thyroids and parathyroids in the neck, in these dogs with so many intrasplenic isoplants, as in the ungrafted dog. Hence we conclude that life was probably little, if at all, prolonged by the absorption of the parathyroids transplanted into the spleen.

RESULTS (*winter of 1907-1908*). The transplantations were made, usually one gland at a time, at intervals of from seven to ten days, behind the rectus abdominis muscle, within its sheath.

Autotransplantation. Of eighteen autotransplantations in 12 dogs, 7 parathyroids were absorbed or necrotic (Dogs 3, 4, 5, and 10); 5 to 7 lived and performed their function (Dogs 1, 7, 8, 9, and 11). What the fate of the four remaining glands would have been (Dogs 2, 3, and 14) is doubtful, the dogs having died of distemper.

Isotransplantation. Of 22 isotransplantations with created deficiency (Dogs 7, 12, 13, 15, 16, 17, 18, 19, 20, 23, and 24), 19 parathyroids were absorbed or necrotic. The result in one instance remains to be determined.

Dog 7, deprived of all parathyroids except the one transplanted, an autograft, lived in good health and spirits twenty-five days, or until, at a final operation, the sustaining parathyroid was removed. There was in this dog, at times, a suggestion of hypoparathyroidism in a barely perceptible fibrillary tremor of the tongue and of the temporal muscles. On removal of the perfectly normal autograft behind the rectus muscle, tetany developed within twenty-four hours and death occurred within forty-eight. Isotransplantation or iso-grafting (two grafts) was unsuccessfully resorted to twenty-four hours after the supervention of tetany.

The Technique of the Transplantations. Usually the superior, but occasionally the inferior, unshaved, parathyroid was selected for the transplantation. In our earlier experiments I shaved one side of the

gland to make a raw surface; this is not always easy of accomplishment, and it is of doubtful value. The graft was transferred with as little delay as possible direct from the neck to its new position behind the rectus muscle. The incision through the rectus sheath, within $\frac{1}{2}$ cm. of the midline of the abdomen, was made only large enough to admit easily the forceps carrying the gland. With a sharp-pointed artery clamp the rectus was deflected outward within its sheath and the parathyroid carried high up behind the muscle, well out of the way of the incision or of a possible serous oozing and the non-demonstrable reaction of the wound. At subsequent operation or at autopsy the situation and condition of the grafts shimmering through the peritoneum and thin transparent fasciæ is instantly ascertained.

Aseptic precautions, thanks to the skilful assistance of Dr. C. W. Hennington, were throughout as perfect as we understand making them. The abdominal wounds all healed per primam. About a graft we have never noted, macroscopically, evidence of reaction. The neck wounds, also with few exceptions, healed throughout per primam. The unusually accurate stitching with the finest black silk, Nos. A to 00, may have contributed in part, I believe, to the unlooked-for perfection in the healing of the neck wounds. Faintly oozing points were carefully included in the continuous sutures, of which there were five or six rows—one each for (1) the midline muscles; (2) the deep fascia and muscle sheath; (3) the platysma and deep layer of superficial fascia; (4) the deep cutis and superficial layer of superficial fascia; (5) the mid-cutis; (6) the epithelium. The epithelial stitch we find very useful in dogs, for it is unnecessary to remove a stitch so superficial as the one thus designated; it, finally, is shed with the epithelium, or it may be peeled off. The sewing consumes very little time, a fine, straight milliner's needle, such as we have used so many years for intestinal suture, is employed.

We have not as yet determined that one tissue or site is better than another for the transplantation, and do not even know that parathyroids deprived of their blood supply in the course of operation or by experimentation may not as well be left undisturbed in the original situation in the neck as transplanted into the thyroid gland or elsewhere. That drainage is usually employed in thyroid lobectomy, and that the drain is usually carried to the precise situation of the para-

thyroids, to the deepest part of the wound, is a fact in favor of the transplantation of the glandules, as into the thyroid isthmus or opposite lobe.

Dr. W. L. Moss has very kindly tested the sera of a number of the dogs used in the experiments. Reactions such as hemolysis and agglutination have not been obtained. Causes of failure in the isotransplantations await discovery. We have in view attempts to dehostilize the foreign tissues by transfusion. An interchange of blood might be accomplished from artery to artery, permitting both hearts to pump into the common arterial system.

SUMMARY. Our experiments have proved that, in dogs—

1. Parathyroid glands are essential to the life of the animals, and that tetany follows their removal.

2. Transplanted parathyroids (autografts) may for an undetermined time perform, at least, the most evident function of these bodies.

3. One successfully transplanted parathyroid may suffice to maintain a fair degree of health, even when traces of hypoparathyroidism persist.

4. In autotransplantation success is more common than failure. And they seem to indicate that

5. Isotransplantation rarely succeeds.

6. For the successful transplantation of these organs a deficiency of parathyroid tissue should be created.

7. Transplanted in excess of what is required by the organism, parathyroid glands probably do not survive.

8. Excised or deprived of its blood supply in the course of an operation, the parathyroid should be reimplanted, preferably, perhaps, into the thyroid gland.

9. Complete excision of the thyroid lobes is well borne, for months at least, by these animals. Myxedema, however, begins to manifest itself within a few weeks.

The Physiology and Pathology of the Parathyroid Glands.

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THE anatomical characters of the parathyroid glands are now well known from the writings of many recent authors, but as to their function there yet remains much that is obscure. Ablation of the glands and the reintroduction of the gland substance are the chief methods which have been employed in this study, but recently chemical investigations have been instituted in connection with them.

The tetany which follows extirpation of the parathyroids has been familiar for a long time, but investigators are only gradually arriving at the conclusion that this tetany may be produced in all animals when the position and number of their parathyroids is sufficiently well known to permit of their complete removal, and, further, that it is dependent solely upon the destruction of the parathyroids and is independent of the thyroid gland. The confusion which has existed in connection with a supposed relation between the thyroid and parathyroid glands is rapidly disappearing. The idea that the parathyroid glands are of no significance, or at most represent merely portions of the thyroid gland which have not as yet become completely mature, but which may, when occasion demands, develop into thyroid tissue, is possibly still held by some English and other observers, but can hardly be taken seriously. The organs are of very different histological character and of different, embryological origin. Their function is shown to be different, and even the chemical similarity in containing iodine in considerable quantity, which was claimed by Gley, has proved erroneous, for it has been shown by Estes and Cecil that the parathyroid glands contain no iodine, or at most an infinitesimal quantity.

The tetany which follows parathyroidectomy is a rapidly fatal affection in dogs, but its course in other animals, such as the rat, is often slower and more protracted. It may be modified experimentally in various ways, and such experiments have been used in order to determine more closely the nature of the condition and the mode of

¹ Read by invitation.

action of the gland. Bleeding, with the infusion of salt solution into the veins, will generally cure the tetany for a time. This afforded the suggestion that there might be a circulating toxin in the blood, which is ordinarily neutralized by the intact parathyroids, but we have never succeeded in producing tetany in another dog by the introduction of blood removed from one in tetany. No direct demonstration of such a toxin can be brought forward in that way.

Tetany may also be cured by the injection subcutaneously or into the veins of an extract or emulsion of the parathyroid glands. The effect of such an injection appears several hours later, and may last a day or two when tetany again appears. Indeed, such a procedure was used to cure the violent tetany which followed the complete or nearly complete extirpation of the thyroid in a little girl (case published elsewhere by Dr. Branham). Several pieces of parathyroid tissue were found adhering to the portions of thyroid removed, and the child, seventy-two hours after the operation, was found in the most typical and violent tetany. Subcutaneous injection of an emulsion of the parathyroid glands of the ox produced a very rapid disappearance of the symptoms, and the child awoke next morning feeling well. Four days later another quite similar attack was similarly cured, since which time she has been well. Apparently some parathyroid tissue was left behind which by this time recovered its normal activities.

Such parathyroid therapy has been quite extensively used recently in different countries in connection with postoperative tetany, and Dr. Halsted has described favorable results in one such case in this country. Indeed, it has been applied, as it is said, with good results in a number of other conditions which are as yet not definitely known to be due to parathyroid insufficiency, such as paralysis agitans, etc. Recently it has been shown by Beebe that it is possible to isolate a portion of the extract of the parathyroid gland by precipitation with acetic acid, which has the essential power of curing the tetany while the remainder not thus precipitated is inert. We have been able to confirm experimentally these results of Beebe's, and find that the nucleoproteid thus precipitated from the emulsion of parathyroid glands will quickly dispel the symptoms of tetany in a parathyroidectomized animal, while the remaining substance is quite without

effect. This material may be dried and kept indefinitely, although it is somewhat difficult to dissolve it, even in alkaline solution, after it has been dried. Such therapeutic methods are, of course, valuable in combating acute and chronic symptoms of tetany, but their use over long periods is accompanied by many difficulties. It is, therefore, most desirable that the technique of transplantation or implantation of the living parathyroid gland should be perfected, so that a permanent relief of the symptoms may be effected, as may be confidently expected for such a procedure.

A number of recent studies which have concerned themselves with calcium metabolism, especially in relation to muscular twitchings, have interested us, and the writer with Voegtlin has carried out experiments concerning a similar relation between calcium metabolism and the tetany of parathyroidectomy. Loeb and J. B. MacCallum observed that muscles treated with salts which were capable of precipitating calcium showed peculiar twitchings, which could be immediately made to disappear by the application of a fresh solution of a calcium salt. Sabbatani, Roncoroni, Regoli, and others observed that the application of calcium salts to the cerebral cortex diminished its irritability. Quest, Stöltzner, and others studied the relation of calcium in the nutriment and in the tissues to the tetany of children, while Erdheim assumes a relation between the parathyroids and osteomalacia, where it is obvious that the calcium metabolism is disturbed. Further, it is stated that animals fed on calcium rich milk do not develop tetany so readily as those otherwise fed. We found that in dogs, in which after parathyroidectomy the most violent tetany had developed with muscular rigidity, clonic spasms, extremely rapid respiration and pulse, etc., all the symptoms can be instantly dispelled by the injection of a solution of a calcium salt into the jugular vein. We have usually used the acetate or lactate, and have given about $\frac{1}{2}$ gram at a dose. The effect of such an injection is marvellous to behold, for sometimes immediately, sometimes after a few minutes, every symptom disappears and the animal returns to a state of complete wellbeing, gets up and runs about, and eats and drinks eagerly. This condition lasts for a day or so until the effect of the calcium wears off, when tetany again appears and may be cured again by a similar dose. Subcutaneous injections, or the introduction of the

calcium into the stomach, act equally well, but very much more slowly. Magnesium seems to stop the tetany, but it completely anesthetizes the animal, and its toxic effects are such that for a time we have had to use artificial respiration to keep it alive. After that it is profoundly depressed and apathetic for a day or two, while the dog treated with calcium is perfectly well. Salts of sodium and potassium seem rather to accentuate the tetany, and when they have been given it seems to require a larger amount of calcium to bring about the cure.

We have made some studies of the excreta which tend to show that during tetany there is an increased output of calcium in the urine. These studies are, as yet, very incomplete, however, but new ones are well advanced, based upon the excreta of animals which have been fed with extreme care.

On the other hand, the analyses of the blood and brain and muscles of a dog dying in tetany have been undertaken, of course, with controls from the normal dog on the same diet. So far the analysis of the blood is complete, and shows a calcium content about half that of the normal dog.

On the whole, then, it seems that the parathyroid glands exercise some sort of control over the calcium metabolism, so that when they are destroyed there arises an increased excretion of calcium possibly associated with imperfect absorption or assimilation of the calcium, so that the tissues quickly become impoverished so far as their calcium content is concerned. Hence the conditions studied by Loeb and J. B. MacCallum are approached, and we can readily understand that the rapid introduction of a fresh solution of a calcium salt will, as in their experiments, tend to stop the twitching. In tetany, however, it has been shown that the twitching is dependent upon an affection of the nervous system and is not merely a local affection of the muscles, for a muscle isolated from nervous control does not twitch during tetany. In some ways the condition is analogous to that seen in diabetes mellitus, where the destruction of the islands of Langerhans interferes with the control of the carbohydrate metabolism, and much carbohydrate is abnormally excreted. Here the loss of the parathyroid allows of a similar rapid excretion of calcium salts. On this idea it seems easy to explain the tetany of lactation as due to the draining of the tissues in the production of the calcium-

rich milk, and the tetany which so often accompanies osteomalacia and rickets may also be connected with the obviously great disorder of the calcium metabolism in those diseases. It is possible that these observations may be of interest from a therapeutic point of view in connection with the various forms of tetany mentioned, that associated with lactation and pregnancy, postoperative tetany, juvenile tetany, gastric tetany, etc. At best, the administration of calcium could have but a temporary effect, but its administration is so simple and free from inconvenience that it may still prove a useful method.

DISCUSSION OF DR. HALSTED'S AND DR. MACCALLUM'S PAPERS.

DR. E. A. SPITZKA. Embryologically considered, the parathyroid bodies are derivatives of the epithelial lining of the gill arches and the gill clefts which become closed in the transition from gill-breathing to lung-breathing animals. In discussing the development of these bodies and their intimate relations with the thyroid gland, I would like to call attention to the morphological changes in the pharyngeal region of the archenteron in general. Thus, the tonsils, most fully developed in mammals, are formed from cell buds of the epithelial lining of the second inner pharyngeal (gill) cleft and later become invaded by lymphocytes. The thyroid is formed from three diverticula, one median and, therefore, unpaired, growing from the region of the copula of the second visceral arch, just caudal of the tuberculum impar at the site of the foramen cecum of the adult tongue. This median outgrowth migrates to a pre-tracheal position (its stalk occasionally persisting as the thyroglossal duct) and is joined by the lateral thyroid buds which develop in like manner from the dorsal wall of the fourth inner pharyngeal cleft. Primarily the organ appears to have been a glandular appendage, whose secretion was of importance in the physiochemistry of the alimentary canal. A later change of function, rather than a regression of the gland, modified the persistent thyroid into an important organ remarkable for its rich vascular supply and the production of a very essential iodine-containing albumin. Yet another important organ, the thymus, arises as a pair of outgrowths from the epithelium of the third cleft, each out-

growth elongating greatly to eventually assume a position ventral of the pericardium. The parathyroid bodies, however, are genetically independent of both thyroid and thymus, and develop in pairs from the third and fourth inner clefts. Their relationship with the thyroid is merely a topographic one, and they may be included in the mass of either thyroid or thymus; their number may be greater than four. Obscure as is their physiological and phylogenetical significance, it is interesting to note that gill-breathers (fishes and larval amphibia) do not possess these epithelial bodies.

DR. JOHN J. GILBRIDE. Without going into details in reference to the anatomy of the parathyroid glands, I wish to say, however, that the position of the parathyroid bodies is subject to a great deal of variation. In dissections made on ten cadavers, at the Medico-Chirurgical College, recently, I found only two instances in which these glands occupied approximately similar positions. The most common site of the parathyroids is in the region of the inferior poles of the thyroid gland, the superior or external glands lying behind and close to the inferior border of the lateral lobes and in close proximity to the inferior thyroid artery; the inferior or internal glandules lying posterior and below the superior glands nearer the median line, or below the thyroid and resting on the anterior lateral surface of the trachea. The distance of the inferior parathyroid below the thyroid, when it occupies that position, also varies, the glands being close up to the inferior border of the thyroid or at a distance of 1, 2, 3, or more cm. below.

Again, both pairs of glands may lie below the inferior border of the thyroid gland by the side of the trachea, and they are not infrequently found at about the centre of the posterior internal surface of the thyroid, the external pair above and the internal pair below the main branch of the inferior thyroid artery, and both pair at about an equal distance from the median line of the neck. The position of the glands varies frequently with the course of the inferior thyroid artery, from which vessel they usually receive their blood supply. Occasionally these bodies are found at or near the respective poles of the thyroid gland, under which circumstances the superior glands may receive their blood supply from the superior thyroid artery. In one specimen of the thyroid and parathyroid that I dissected there was an anoma-

lous condition of the superior thyroid arteries. The right one being absent, the left superior thyroid artery was rather large and passed across the superior border of the thyroid, supplying the whole of the upper part of the gland on both sides with blood. In this instance there were three parathyroid bodies. The right superior gland being absent, the left superior body was nearly three times the size of the average gland, while the two inferior bodies were of average size.

As to the number of these bodies, in one cadaver I found four on one side, the right, and two on the left. There were three of average size on the right side and one small body about the size of a mustard seed. The two glands on the left side were of average size. There was also one interesting point that I observed: in a few instances one-third or one-half of the gland was of a light-yellow, fatty tissue, with a distinct line of demarcation separating it from the darker brown color of the rest of the gland. No microscopic examination was made of these glands, but the yellow and the brown parts of the body were enclosed in the same capsule and appeared together to form the gland. Whether this condition is of any significance I do not know.

DR. NATE GINSBURG. The position and blood supply of the parathyroid bodies of the specimens exhibited conform in a general way to the description of writers on this subject. One parathyroid body is of interest, mainly because of its unusual position and blood supply. Contrary to what is commonly observed, one of the superior parathyroids derives its blood supply from the superior thyroid artery instead of from the inferior thyroid artery. The artery is of unusual length and is distinctly isolated. On the same specimen the left inferior parathyroid body will be found quite deeply embedded in a recess in the gland substance, apparently a part of the gland, but easily isolated and removed. These variations are not uncommon, and occur in a small proportion of cases.

DR. H. A. HARE. The points which have seemed to me to be most important are, first, those which have to deal with the vascular supply of the parathyroid bodies, because this vascular supply is a guide to the glands and must be cared for lest it be damaged, and, second, with the fact that the parathyroids are often embedded in the thyroid gland in such a manner that they may be readily overlooked, particu-

larly as their relative positions in respect to the geographical markings of the thyroid are variable. Thus, they are sometimes found on the isthmus of the thyroid. The fact that there may be supernumerary parathyroids may explain why it is that tetany parathyropriva fails to develop in certain cases after these bodies have been damaged or removed, and in some patients a considerable number of these bodies may be found in widely separated positions. Thus, in Pool's 16 dissections the average number of parathyroids was 2.9 per person, but in Verbely's statistics he found 4 parathyroids 108 times in 138 autopsies. Getzowa has found accessory parathyroids within the thymus or in the tissues below the thyroid. Again, the interesting studies of Forsyth upon no less than 50 human and 70 different species of animals and birds show a marked variation in number. Thus, in 3 monkeys the parathyroids numbered 1, 8, and 4, respectively, and further, sometimes a parathyroid was found embedded in a lymph node and was microscopic in size.

In this connection, too, we must consider the somewhat hypothetical proposition that aggregations of cells capable of performing parathyroid functions may exist in other parts of the body, just as Zuckerkandl's parasympathetic bodies, Luschka's coccygeal gland, and the intercarotid gland (Mulon) have been found to contain cells which are supposed to perform a function identical with the medullary portions of the suprarenals. Forsyth has collected several such cases.

The belief that the parathyroids are widely different in function from the thyroids is vigorously combated by Forsyth, who believes them to be part of the latter, or "splittings off" which have assumed functions, but have not yet formed vesicles. He thinks that the thyroids and parathyroids secrete the same substance. He believes that the parathyroids are immature thyroids, and advances several reasons for his belief.

It is evident, therefore, that a number of factors enter into the problem of parathyropriva cases. The following questions suggest themselves:

Since such good results follow the injection of glycerinated extract of parathyroid gland, why resort to transplantation, which has not been successful with thyroids?

What evidence is there that the spleen is the best area for trans-

plantation when we know that the function of very vascular networks is to destroy animal and vegetable complex substances?

In rickets there is an excess of calcium in the urine due to lack of absorption. Is the same true in parathyropriva?

DR. JOHN B. DEEVER. That the subject of discussion is an important one no one would deny in the light of the valuable work that has been accomplished along these lines. That the successful outcome of any operation upon the thyroid gland depends either upon recognition of the parathyroids or certainly non-interference with these little bodies we must admit.

I attribute the absence of tetany in the cases upon which I have operated to the fact that I have not trespassed upon these bodies; while I have recognized them latterly in one or two cases, in the majority of cases I have not. It has always been my practice to dissect close to the goitre, hugging it, as it were, with the scalpel at every step of the dissection, working within the posterior capsule, which I dispose of with gauze. It is always my experience that the capsule of the thyroid gland in pathological conditions is much more distinct than in the normal gland, I have had many opportunities of proving this.

The incision which I always make in this operation is the so-called collar incision. The only death that I have had following the operation for simple goitre was due to hyperthyroidism, thyroid toxemia, which immediately followed the operation. The temperature as well as pulse became high, the patient becoming delirious and dying on the second or third day; there was never any evidence of tetany, however.

Death after operation for exophthalmic goitre has not occurred until three weeks following the operation for removal. The patient simply dropped over dead from, I think, dilatation of the heart.

DR. CHARLES FRAZIER. Our interest in parathyroid function has been aroused by the occurrence of tetany as a complication or sequel of thyroidectomy, and I am sure Dr. Halsted, through his work and writings, is largely responsible for attracting the surgeon's attention to this very important feature of the surgery of the thyroid gland. I was led to believe that tetany was occurring with alarming frequency even in the hands of experienced surgeons. Inasmuch as my experience and the experience of my colleagues at the University

Hospital did not bear out this statement, I was prompted to ascertain from the leading surgeons of the country whether or not my experience was unique. In the experience of 54 surgeons there had been only 8 cases of tetany; of these, 3 were fatal, 1 having died two years after the operation with symptoms only suggestive of tetanus. Of the remaining 5, 1 was transitory and 1 was described as a slight case. The total number of operations upon the thyroid gland represented in these statistics is between 1500 and 2000 cases. Granting that many of the cases in this series were partial, not complete thyroidec-tomies, with a total of only 8 cases, it would appear as though the frequency of tetany as a sequel of thyroidectomy is not such as to occasion much alarm. Of this series of 1500 to 2000 cases, C. J. Mayo performed 560 operations, with but 1 slight case; Crile, 160 operations, with no cases; and McCosh and Sheppard, 150 operations each, with no cases.

Another point which is of practical interest in the consideration of this subject is the recognition of the parathyroid glandules in the course of the operations. My own experience, based on observations at the operating table and in the pathological and anatomical laboratories, prompts me to question the feasibility of recognizing the parathyroid glandules with any certainty as a routine procedure. Even at the close of an operation I have at my leisure, and unembarassed by a blood-stained field, searched in vain for the glandules upon the specimens removed. I find that my experience is rather in accord with that of the majority of surgeons. Of 54 surgeons who answered my inquiry regarding the possibility of recognizing these glandules, only one spoke confidently, or at least positively, as to his ability to recognize these bodies. In his series of 160 operations, although looking carefully for them, Crile has only been able to identify them in 3. The most important feature of this whole subject, at least from the stand-point of the practical surgeon, is the development of a technique designed to preserve the integrity of these glandules. There is no question but, theoretically at least, the subcapsular operation is the operation of choice, but I should like to ask Dr. Halsted whether he has not found in many instances this operation difficult of performance; difficult in the recognition of the capsule and difficult in the attempt to strip the capsule from the gland. Owing to the

fact that the capsule gives off septa, which penetrate the glandular substance, it is difficult to remove the capsule without removing with it a thin layer of parathyroid tissue. The latter modification of the technique was first suggested by Kocher.

Even if the parathyroid glandules should be preserved, some means must be adopted, of course, to preserve their blood supply. The suggestion which Dr. Halsted makes, that in ligating the inferior thyroid artery the clamp be plunged into the substance of the gland, seizing the artery after it has disappeared from view, is an excellent one. A somewhat safer method, it seems to me, is that of Pool, that at least one of the thyroid vessels, preferably the inferior thyroid, should not be ligated. This suggestion has proved to be of greater significance since Ginsburg (*University of Penna. Med. Bull.*, January, 1908) published his observations on the blood supply of the parathyroid glandules. Ginsburg found, what escaped the notice of previous observers, that there is a secondary or accessory blood supply to these small bodies. In other words, that there is an anastomosis between the parathyroid arteries on one side with those of the other, so that if one of the inferior or superior thyroid arteries is left intact the blood supply not only of the parathyroid bodies on one side, but of those on the other is preserved. As to the relative importance of the arterial trunks, the inferior thyroid artery is the more important, because the parathyroid artery takes its origin in the majority of cases from this vessel. The existence of this anastomotic relation between the vessels of either lobe no doubt accounts for the fact that tetany has not developed in a larger percentage of cases after thyroidectomies. Many of the 1500 to 2000 operations referred to in this report were performed before surgeons were aware of the existence, the function, the minute anatomy, or the vascular supply of parathyroid glandules. Unless some such explanation could be offered, it would be difficult to account for the comparatively insignificant number of cases of tetany that have occurred in the practice of so many experienced surgeons. In the existence of accessory parathyroids we have another explanation for the comparative infrequency of tetany. These accessory glandules are found in positions which the accessory thyroids are likely to occupy, or, as in a case reported by Erdheim, in which two small accessory glandules were found in the thymus.

DR. J. CHALMERS DACOSTA said that very few tumors of the parathyroid have been reported. It is probable that in some cases the condition has not been identified. Nevertheless, it is certain that tumors of this gland are extremely rare.

His patient was a white female, aged thirty-two years, on whom he operated October 29, 1906. There was nothing peculiar in the history except continued menstrual disorders. At twenty-eight years of age she was married, and two years later was operated on for appendicitis, at a time when she was three months pregnant. Four weeks after the operation she miscarried.

Seven years before admission she had had acute tonsilitis, with abscess formation in each tonsil; and six months after the attack of tonsilitis she noticed a swelling on the right side of the neck. This swelling gradually increased in size, and began to increase rapidly after the performance of the abdominal operation. It was free from pain, but she had considerable dyspnea on exertion. If, while lying down, she attempted to lift her head, she suffered with a severe choking sensation.

She was thin, neurotic, and pallid. The pulse was strong, and 72 a minute. The tumor was right-sided, and was overlapped by the sternomastoid muscle. It passed down back of the clavicle, and reached above to within 1 cm. of the upper border of the thyroid cartilage. It moved with the trachea on swallowing.

It was regarded as an adenomatous goitre, and was removed under local anesthesia. Great difficulty was encountered in reaching and freeing the lower pole. The inferior thyroid seemed to be anomalously distributed, and was found in front of the tumor, rather than at its lower and inner side. Great difficulty was experienced in separating the recurrent laryngeal nerve, which was distinctly visible.

No parathyroid was observed during the operation. The tumor was irregular in outline, and presented, in front and at the lower portion, a bulb-like projection, somewhat darker and softer than the remainder of the mass, but apparently a part of it. The bulk of the tumor was brownish yellow.

Recovery was prompt and without unfavorable symptoms. The patient was discharged, apparently well, nine days after the operation.

Even after having removed the growth, Dr. DaCosta had no sus-

picion of its being parathyroid, and did not know this until the pathologist's report was submitted to him. That report showed the tumor to have been a hyperplasia or an adenoma of the parathyroid. The bulk of the mass was parathyroid, and the bulb-like projection was thyroid.

Dr. DaCosta then referred to the reported cases of parathyroid tumor, there seeming to have been seven certain and two doubtful instances. In practically all there was uncertainty as to whether the condition was hyperplasia or adenoma.

DR. A. P. BRUBAKER. The experiments of Vincent and Jolly are of interest in this discussion. They were published in *The English Journal of Physiology* in 1904 and 1906. These experiments were made on cats, dogs, monkeys, rats, guinea-pigs, foxes, badgers, wolves, and rabbits. As a rule, both the thyroid and the four parathyroids were removed. Notwithstanding expectations, many of the animals thus operated on lived for several months without manifesting in a marked degree any of the usual symptoms attributed to parathyroidectomy. Then they state, in summing up the results of their experiments, that more than 51 per cent. survived the operation for a prolonged period, and of these more than 68 per cent. showed no specific symptoms of any kind. In those animals killed after a prolonged period following the operation, it was impossible to discover any parathyroid tissue. They conclude that it cannot be truly said that either thyroids or parathyroids are absolutely essential for life.

The histological findings are also worthy of note. In the parathyroids of the cat, after removal of the thyroid, changes have been found which indicate that the parathyroid tissue approximates in appearance thyroid tissue, so that finally they become indistinguishable, after which the parathyroids functionate in place of the thyroids.

DR. HALSTED: I am very much interested to learn from Dr. Frazier that so many unilateral lobectomies have been done in this country, with so few cases of hypoparathyroidism. But it is only from operations on both lobes that we particularly fear tetany; and for the absolute cure of exophthalmic goitre the single lobectomy is often, I might say usually, insufficient. The prospect or possibility of an operation upon the second side must always be borne in mind* in the course of operation upon the first. I do not wish to give the impression that I

advocate the excision of more than one lobe in the majority of cases of Graves' disease. That I do not is rather because, hoping for some therapeutic measure less barbarous than the operative one, I dislike to deprive the individual of an organ as to the value or function of which we are still quite ignorant.

In reply to Dr. Hare, I would state that (1) the frequent or daily injections of extracts of the parathyroid gland would, even if possible, be most irksome to the patient. The services of a physician or nurse might be essential, and, notwithstanding the greatest precautions, occasional infections would result. The extract which, thanks to Dr. Beebe, we have at present deteriorates in a few weeks and is difficult to furnish. But aside from the above and other considerations which might be mentioned, how incomparably simpler and better is the trivial operation of transplanting one parathyroid gland than the use of a hypodermic syringe or even medication for a lifetime. (2) There is not, as yet, conclusive evidence that the spleen is the best place for the transplantation. We are not sure that it is at all better than other organs or situations.

Bacterial Vaccines of Staphylococcic Strains. A Technique for their Preparation.

By HARLAN SHOEMAKER, M.D.

I HAVE been enabled to make the following observations while at work on the immunization of cases of furunculosis, acne, and sycosis in Prof. J. F. Schamberg's clinic for diseases of the skin at the Polyclinic Hospital. The work has been done in the laboratories attached to the hospital with the kind permission of the registrar, Miss Kirkbride.

The experimental evidence of phagocytosis by Metchnikoff and of bacteriolysis by Pfeiffer as a chain in the mechanism of immunity has been elaborated by the French and German schools; and Wright and his followers have placed the former theory well before the profession.

It is my pleasure to bring before this Society for discussion a state-

ment of the effects produced by the application of heat upon the bacterial body, as to the degree of intensity, duration of exposure, and the relation this bears to the immunity conferred by inoculations of bacterial suspensions so prepared.

A number of the workers in this country and abroad have dismissed this subject with a word. They claim that their bacterial suspensions are "killed cultures" rendered incapable of further propagation. Sir Almoth E. Wright (*Lancet*, 1902) first killed his cultures at 65° C. for twenty minutes. One patient, of a series of 6 reported in this paper, developed a localized inflammatory reaction at the point of inoculation. Wright asserts that this result occurred from staphylococcic matter already in the system of the patient; all 6 cases, however, showed evidence of pronounced local reaction, as well as constitutional symptoms. Subsequently, Wright (*Proc. Royal Soc. Lond.*, July 26, 1904, p. 154) found it necessary to keep the suspension of bacteria in the incubator twenty-four hours after heating at 60° C. for thirty minutes. The suspension was then cultured and at the same time enumerated; 25,000,000 to 75,000,000 bacteria were used. Should the culture media show evidence of bacterial growth, the technique was repeated until the organisms had lost their power of further multiplication.

E. H. Schordor, of Rockefeller Institute, makes a reference to the use of killed cultures of streptococci, employing 25,000,000 to 100,000,000. Dr. Simon, of Baltimore (*Exp. Med.*, September 21, 1907), in a paper read before the Association of American Physicians last assembled in Washington, D. C., makes the most remarkable statement that he can see no difference in the reaction of a patient to his vaccine whether 25,000,000 or 1,000,000,000,000 staphylococci are given. And Wright and Reed, when employing the colon bacillus for immunization in cases of cystitis, make use of a sterilized vaccine containing 200,000,000 organisms.

Since we find such a variety of ideas and conclusions among the workers, apparently the elaborate experiments in immunity present the only analogy to the effects of heat on a bacterial body. Here one is confronted by great diversity of terminology, but finds, on the other hand, a marvellous unanimity of results.

Regarding the various theories elaborated from the standpoint

of temperature alone, there has been found in the blood a substance thermostabile at 60° C., and a substance thermolabile at the same temperature. Ehrlich and the German school express an amboceptor stabile at 60° C. and a complement labile at 60° C. Bordet and the French school designate their thermostabile and thermolabile products as fixateur and substance sensibilisatrice, respectively.

Bordet (*Annal. Pasteur Inst.*, vol. xii, No. 10) has recently shown the analogy between hemolysins and bacteriolysins. Both substances are thermolabile, and if they be destroyed by heat the serum will still be found to contain a thermostabile substance which has the power of agglutinating either the blood corpuscles or the bacteria, as the case may be. This latter substance of a thermostabile nature in the serum he terms a fixateur.

Wright and his followers determine an incitor stabile at 60° C., and an opsonin labile at the same temperature. Wright (*Proc. Roy. Soc. Lond.*, vol. lxxvii, Series B) invents the term incitor to explain the phenomena of phagocytosis of bacteria by washed leukocytes in the presence of blood serum which has been heated for ten minutes at 60° C. Although the opsonin has been removed from the serum by heating, there yet remains a substance of a thermostabile quality, which unites with the bodies of the bacteria. They are rendered capable of phagocytosis by the incitor without the presence of opsonin or any other thermolabile product in the serum. Wright does not admit the presence of any new body in the blood serum, notwithstanding his use of the term incitor, and claims that the thermostabile substance does not exist, there being instead an opsonin attenuated by heat, thereby disproving any possibility of spontaneous phagocytosis.

Dean (*Proc. Roy. Soc. Lond.*, July 8, 1905, Series B) produced an active phagocytosis of bacteria specially treated, in which the presence of an immune serum was displaced by a physiological salt solution. The bacteria used in the experiment were centrifuged through a heated immune serum, and thoroughly washed free of the serum before being used. It was found that the organisms selected something from the immune serum which rendered them capable of being engulfed by the white blood cell. This serum is quite incapable of producing phagocytosis in the usual way, with the species of bacteria

used, although it is active in opsonizing any other species of bacteria.

Two years later Cowie and Chapin (*Jour. Med. Research*, 1907, vol. xii, No. 1), in experiments similar to those of Dean, and independent of any knowledge of his work, arrived at the same conclusions, and Wright (*Lancet*, Nov. 2, 1907) adds a beautiful confirmatory analogy to this work when he demonstrates the difference between the opsonic power in the normal blood serum and in the serum obtained from the focus of infection. The latter is very deficient in opsonin.

Regardless of the attempts of the German school to explain immunity by a multiplicity and specificity of each amboceptor and complement, or those of the French school to confine all serum reactions to a duplex role, the various experiments conform alike to heat exposure. Wright does not accept this, although he readily admits the modification of the blood serum by heat, while, judged by the variety of their terminology, his technique in the hands of competent observers in this country and abroad shows opsonin to hold the same relations to temperature as those which are expressed in their experiments.

	<i>Substances in blood serum thermostabile at 60° C.</i>	<i>Substances in blood serum thermolabile at 60° C.</i>
Ehrlich and German School	Amboceptor.	Complement.
Bordet and French School	Fixateur.	Substance sensibilisatrice.
Wright and English School	Incitor.	Opsonin.

SUMMARY OF OBSERVATIONS.

(a) Opsonin in the serum is modified by heat.

(b) Bacteria are incited to phagocytosis by serum from which the opsonin has been removed by heat.

(c) Bacteria unite with a substance in heated serum which prepares them for phagocytosis.

(d) Bacteria remove a substance from the serum collected at the foci of infection, which reduces the opsonizing power of this serum, for this species and no other, below that of the patient's blood serum.

These observations deduced from actual experimentation do not prove the presence of a thermostabile and a thermolabile substance in the bacterial body, but they suggest it. The bacterial body differs

from the blood serum, perhaps, in the molecular arrangement of its elements and in its inherent ability to reproduce itself. This difference presents a step from an unorganized organic substance in the serum toward an organized organic substance in the bacteria.

In the Spring of 1907 Gildersleeve, of the University of Pennsylvania, successfully inoculated 2 cases of sycosis in the clinic. Both patients had constitutional and well-marked local reactions. An abscess developed at the site of inoculation in the second case, while only a cellulitis appeared upon incision of the local tumefaction in the first case. Both recovered completely from the sycosis.

When I first prepared a vaccine, taking 80° C. as the thermal death point of the staphylococcic strains, the constitutional reaction upon inoculation was mild. No local reaction occurred at the site of injection, and very little activity was demonstrable by Wright's method of estimating the phagocytic power of the blood. In all, 16 cases were determined. In these the failure of Wright's method to yield results was attributed to poor technique and insufficient experience.

Subsequently, during the preparation of a vaccine for use in the clinic, the water bath in which the cultures were killed attained a temperature of 90° C. One of the vaccines in this brew was used upon a physician suffering from furunculosis; 40,000,000 staphylococci at 80° C. had caused him some symptoms of constitutional reaction, such as thirst and restlessness at night, accompanied by irregular fluctuations in the opsonic index (0.75 to 1.3 were noted). If previous inoculations of bacterial suspensions prepared at 80° C. were insignificant and but mildly effective, one at 90° C. would do no harm. This vaccine contained 400,000,000 staphylococci. According to the patient, who was a competent observer, this inoculation produced no more clinical effect than the injection of so much coagulated egg albumin; neither did the opsonic index fluctuate, but remained at 0.8.

This shows how it might be possible to give in a suspension 1,000,000,000,000 bacteria subcutaneously inoculated, without untoward effect.

Now I began a gradual reduction in the temperature applied to the water bath. The inoculation of a bacterial suspension, heated at

70° C. for one-half hour, gave evidence of fluctuation in the phagocytic index; at 65° C. for half an hour, produced more pronounced constitutional symptoms; at 60° C. the cultures had to be maintained in the water bath one hour before the thermal death point inhibiting the reproduction of the species was reached. Upon inoculation with this suspension, a violent constitutional reaction was observed. Three strong men who had received injections not only felt feverish and restless the next day, but remained in bed more or less prostrated. Only a few phagocytic counts were made in these cases. They showed that the positive and negative phases in Wright's experiments were well marked by a fluctuation from 0.5 to 3.5, 1.0 being the normal or control index.

We see that the clinical reaction and laboratory phenomena exhibit an increased intensity as the temperature applied to the bacterial suspensions is reduced. The duration of the application of heat is also an element to be considered.

Further reduction of the temperature to 59° C., and then to 58° C., used in the preparation of the vaccines was followed by disastrous results. Five people were unconsciously inoculated with living cultures of bacteria, of autogenous strains, 20,000,000 to 40,000,000 strong, pasteurized for one hour at 58° C. All developed a local tumefaction of brawny induration, with a sensation of deep fluctuation in each case. In 3 of these cases the local reaction disappeared, while 2 patients developed abscesses at the site of inoculation. One of the latter showed a pronounced rise and then a depression of the phagocytic count; the other, at two different readings of several days' interval, exhibited a continued depression of the phagocytic power of the blood. A third case of this series, having relapsed clinically, was re-inoculated with a bacterial suspension treated in a water bath for one hour at 59° C. He developed a small abscess at the site of injection. One reading subsequent to the evacuation of the abscess showed a very high index. From all three cases there was recovered the same organism which had been introduced.

The following is a technique recommended for the purpose of producing the highest "positive phase" of Wright. Prepare:

1. Sterilized glass pipettes.
2. Sterilized test-tubes, each containing small glass beads or sand.

3. A twenty-four hour culture of bacteria of autogenous strain (not necessarily a pure strain) on a nutrient agar slant.
4. A sterile salt solution, 0.85 per cent. NaCl.
5. A water bath.
6. A thermometer.

With a sterile gum attached to the pipette, draw up about 1 c.c. of the salt solution. This solution is played over the surface of the agar slant culture media until the twenty-four-hour old growth of bacteria has been washed free from its surface.

The bacterial suspension is then drawn up into the pipette and immediately transferred to the test-tube containing the glass beads. The tube is now stoppered with its cotton plug and agitated freely, in order to disintegrate the colonies and clumps of bacteria. Having previously heated the water bath to the desired temperature, the bacterial suspension is inserted into it. The surface of the water in the bath should be above that of the salt solution containing the suspended bacteria, or, better still, if the vaccine is sealed in a glass receptacle, complete submersion in the bath is desirable. The thermometer should be kept in the bath under the same relative conditions as those surrounding the bacteria. For example, the bacteria being in a certain quantity of solution in a test-tube, the thermometer should also be similarly placed. Both tubes should then be kept in the closest contact in the water bath. A temperature of 58° to 59° C., or the lowest possible point required to destroy the reproductive activity of the germ, is maintained for one hour. It is then subcultured and enumerated, when the suspensions are placed in the incubator at 37° C. for the remaining twenty-three hours. Prior to a repetition of this technique, which is advised for at least three successive days, a daily subculture following the pasteurization is made from the bacterial suspension. If at the end of the fourth day there is no growth upon the subcultures, and especially the last one, the vaccine may be used for subcutaneous inoculation.

A suspension containing 5,000,000 to 10,000,000 of bacteria prepared at this low temperature is equal to a 400,000,000 bacterial suspension prepared at a higher temperature.

At some point between the temperature necessary for greatest bacterial growth and that for reproductive death of the germ lies a

substance which is active in producing immunity. This substance may be entirely inactivated by heat, or it may evidence marked activity just above the degree of temperature where the reproductive powers of the organisms cease.

The ultimate chemistry of the proteid body remains for the future. Our object is a bacterial product of efficiency which may be injected with impunity. Perhaps this problem may best be solved without heat at all. Digestion by an active ferment and precipitation of the globulins, albumoses and other cleavage products of the bacterial body will help to elucidate this subject (Halliburton, *Proc. Path. Soc. Lond.*, 1905, vol. lvi, p. 158).

When we consider that every surgical wound made for the relief of infected parts gives rise to inoculation of living bacteria and their products, uncontrolled and unlimited; that every massage, active or passive, of diseased areas produces much the same results with absorption of fixed tissue cells and tissue juices in addition, there may be some extenuation for these unusual experiments.

CONCLUSIONS. A thermolabile and a thermostabile substance are found in the bodies of the staphylococcic strains of bacteria, which substances, in the organic bacterial bodies, are of the nature of an amboceptor and a complement.

The activity of the thermolabile moiety of the bacterial body varies inversely as the degree of heat and its time of application.

January 9, 1908.

A Study in Vitro of Liver Necroses Produced by Intravenous Injection of Ether.

A PRELIMINARY REPORT.

BY LEO LOEB AND MILTON K. MEYERS.

IN former experiments on the effect of the intravenous injection of ether in rabbits one of us¹ has shown that such an injection is followed by the formation of thrombi which are typical fibrin thrombi, and not, as has been maintained, thrombi consisting of precipitated proteid material or of agglutinated red blood corpuscles.

The necroses found in the liver after injection of ether into a mesenteric vein were shown to originate in every case in the portal areas; they were first visible one to two hours after the injection, they were somewhat larger twenty to twenty-four hours later, and they increased considerably in the course of the next two weeks.

The necroses were shown not to depend, at least during the first day, upon the formation of thrombi in the vessels, but to be due to a direct toxic action of the ether upon the liver cells.

The following experiments were undertaken with a twofold purpose. In the first place, we wished to carry further our analysis of the mode of action of ether on living vertebrate tissue; and secondly, we desired to undertake an examination *in vitro* of tissue changes which thus far have only been studied *in vivo*.

In contradistinction to the processes of autolysis which take place in excised tissues, the degenerative changes which are caused by certain toxic substances in the body have, so far as we are aware, never been studied *in vitro*. By investigating tissue changes outside the body, we will be able to vary the external conditions under which these changes take place, and this procedure alone will enable us to analyze the different factors concerned in degenerative processes. This method promises, therefore, to become a desirable addition to our means of investigation.

The following consideration was the starting point for our experiments:

If tissues degenerate after they have been cut off from the circulation, the changes which take place are of a twofold nature. In the first place, certain processes occur in the protoplasm which prevent the normal enzymatic activities, and perhaps certain other metabolic changes in the cell. As a consequence of these primary changes the equilibrium of the cell metabolism is disturbed, and certain enzymatic processes, especially hydrolyses, begin to prevail, and lead to the structural changes found in necrotic tissue.

If such changes are caused by interference with the circulation, they take place rather slowly; they are much more rapid, however, as a result of the intravenous injection of ether.

We assume, in a preliminary way, that ether has two effects on the tissues: It eliminates certain, especially anabolic, cell activities, and

enables the destructive enzymatic actions to proceed. If tissue necroses are seen so much more rapidly after intravenous injections of ether than after interference with the blood circulation, the injection of ether accelerates, perhaps, the first set of factors, namely, that which leads to the suspension of the normal chemical cell activities. Afterward the processes of autolysis take their usual course.

We intended to test this hypothetical conception in the following way: We subjected the liver tissue first to the influence of ether *in vivo* by injecting this substance in the usual way into a mesenteric vein, thus allowing the ether to act a short but sufficient time upon the liver cells.

The time during which the ether acted directly *in vivo* upon the liver cells was in no case sufficient to produce even the beginning of a necrosis. In the majority of cases distinct necroses appeared only approximately one and one-half hours after injection of the ether. In our experiments pieces of liver were excised usually ten minutes after the injection. We then subjected the excised pieces *in vitro* to various external conditions which are known to accelerate or to retard the ordinary processes of autolysis. In this way we intended to determine whether, in the course of the ether necroses, external factors act in a similar way upon the ether necroses and upon ordinary autolytic changes.

In the following we state a few of the results obtained. In a later communication a more detailed account of these and of additional investigations will be published.

1. Do periportal necroses take place *in vitro* after ether has been injected intravenously in the living animal? Our experiments permit us to answer this question decisively. In the pieces which have been removed very soon after the injection of ether, and which have been kept outside the body for the next few hours, *the typical periportal necroses are seen to develop with approximately the same velocity as in vivo.*

The morphological character of the necroses *in vitro* is identical with those *in vivo*. The cytoplasm takes up less stain, becomes more or less dissolved, the nuclei shrink, or chromatolysis takes place in other nuclei which retain their normal size; frequently the nuclei disappear entirely in the direct neighborhood of the portal vessels.

This periportal area may be surrounded by a peripheral zone of shrunken cells with pyknotic or chromatolytic nuclei.

2. These facts having been established, we next studied the effect of changes in temperature to which the liver pieces were subjected *in vitro*. We know that most enzymatic processes, in common with other processes of a chemical nature, are, to a considerable degree, influenced by the temperature at which they take place. Near freezing point a marked decrease in the intensity of the majority of enzyme actions takes place.

On the other hand, it might be possible that ether kills the cells directly, and that between the injections of ether and the appearance of the necrosis is merely the time necessary for the washing out of cell protoplasm and of the nuclear chromatin. In the latter case we would have to deal with a purely physical process, which would not be influenced to any appreciable extent by a considerable lowering of the temperature.

In order to decide this question we carried out seven experiments in which the excised pieces of liver were kept surrounded by melting ice during a period of five hours. Control pieces were kept in the thermostat at body temperature. The microscopic examination of the pieces thus treated showed that even the temperature of melting ice did not entirely prevent the necroses; but in most cases the necroses were markedly smaller, and they were invariably less intense than in pieces which had been kept in the thermostat during an equal period of time. An exact quantitative estimation of the effects of various temperatures on the development of the necroses seems at present impossible; but the differences we observed were in most cases so well marked that we may conclude that degenerative changes taking place in tissues after injection of ether are of a chemical, and probably of an enzymatic, character.

3. Several investigators (Baer and Loeb²) have shown by chemical analysis that the process of autolysis is retarded if the tissue is suspended in blood serum instead of in an 0.85 per cent. salt solution, and Wells³ and Longcope⁴ observed that the inhibiting effect of blood serum on tissue autolysis can also be demonstrated microscopically, inasmuch as the histological character of tissues is better preserved in serum than in salt solution.

In a series of experiments we determined whether blood serum has inhibiting action upon the development of the periportal ether necroses. After an injection of ether excised pieces of liver were, therefore, kept in 0.85 per cent. NaCl solution, in defibrinated dog's blood, and other pieces were introduced into the peritoneal cavity of living rabbits. The different pieces were investigated microscopically. The development of the periportal necroses proceeded in a similar way in 0.85 per cent. NaCl solution, in defibrinated dog blood, and in the peritoneal cavity.

4. We have seen that during the first five hours no marked difference exists between the rate of development of the periportal necroses *in vitro* and *vivo*, and we therefore know that *sodium chloride itself has no specific influence upon the development of these necroses.*

5. Toward the end of the first twenty-four hours, however, differences begin to develop in the size and intensity of the necroses in pieces which were kept *in vitro* and of the necroses which were produced in the livers of living animals. Later than five hours after the injection of ether no further change takes place in the size and in the intensity of the necroses *in vitro*. At the end of the first day the necroses are decidedly larger and more intensive *in vivo* than *in vitro*.

6. How is this gradual increase in the size and intensity of the necroses *in vivo* to be explained?

The principal difference between the necroses *in vivo* and *vitro* consists in the gradual accumulation of leukocytes in the area of the peripheral necroses *in vivo*; it is absent *in vitro*.

Leukocytes begin to accumulate in the capillaries of the necrosed areas in the course of the first few hours. At later periods they are not only present in the capillaries, but they penetrate into the liver cells, and here they exert their proteolytic action, although in different necrotic areas the number of leukocytes present varies. Chemical substances which exert a negatively chemotropic influence are certainly not present in these necrotic areas. We emphasize this last because it has been suggested that the reason why aseptic infarcts are so slowly invaded by leukocytes is due to the presence of negatively chemotropic substances in aseptic necrotic areas.

The presence of leukocytes and their activity in the living animal

is responsible for the greater intensity of the necroses *in vivo* at the end of the first day. The leukocytes attack and gradually destroy liver cells situated in the periphery of the necrotic areas, and they may dissolve cells which are more or less injured, even if their histological structure does not yet indicate a complete necrosis.

7. The gradual increase in the size and intensity of the necroses in the living animal cannot, however, be explained as due to the actions of the leukocytes, this increase in the size of the necroses taking place without a corresponding increase in the number of leukocytes, which latter begin to disintegrate at this period. The further extension of the necroses is in all probability due to the presence of the fibrin thrombi that formed under the influence of the ether, and to their possible extension and the consequent interference with the circulation of the blood.

(These experiments are continued in various directions.)

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The Production of Deciduomata¹ and the Relation between the Ovaries and the Formation of the Decidua.

By LEO LOEB.

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LAST summer I published² a short note in which I announced that, if incisions are made into the uterus of guinea-pigs at certain early stages of pregnancy, nodules will be formed that have the structure of the decidua of the guinea-pig. Since then these investigations have been continued. In the following I wish to summarize briefly some of the main results obtained:

1. The operative procedure consisted in making transverse and longitudinal cuts in the uterus of the anesthetized animal. In order to obtain a large number of deciduomata, it is necessary to make many cuts that advantageously separate the continuity of the uterus in different directions. Under these conditions, it is possible to obtain a very large number of deciduomata in the uterus of the guinea-pig.

2. The different stages in the development of these deciduomata were established by microscopic examination. In a number of cases serial sections of the nodules were made. These nodules originate

¹The term "deciduoma" implies that the new formation consists of decidua-like tissue. Those tumors which formerly had been called deciduoma malignum are now almost universally designated chorion epithelioma malignum.

²Centralblatt f. allgem. Pathologie, 1907, vol. xviii, No. 14.

through a proliferation of the interglandular connective tissue of the mucosa. The proliferation takes place through mitotic cell division. Occasionally, apparent amitoses can be seen. At first, a mass of myxoid tissue is formed underneath the epithelium of the mucosa. This newly formed layer is very rich in blood capillaries, but extremely deficient in glandular structures. The glands of the uterus participate in this proliferation not at all or only to a very slight degree.

As early as twenty-four hours after the operation it is possible to observe the beginning of the proliferative changes. On the third day small myxoid nodules are present, with many mitoses. The size of the cells, especially directly beneath the epithelial layer, is markedly increased. During the following days the cell proliferation continues. After five days, small decidual nodules have formed, with many mitoses, which are also present in the endothelium of the bloodvessels. The cells beneath the epithelium are very large, but have not yet attained their full size. As in the earlier stages, the bloodvessels run at right angles to the myxoid cells. The deciduomata reach their full size ten to eleven days after the operation. Mitoses are still present at that time, but are not so frequent as before. They are usually found not in the large cells, but in foci of small cells. The cells at this stage have commonly increased very much in size, and resemble the decidual cells of the normal placenta. The superficial part of the mucosa frequently becomes necrotic under the influence of the pressure of the nodule, and is cast off. At other places we find inclusions of the surface epithelium in the tumor. This is due to an occasional papillomatous condition of the growth. The epithelium between two papillae may be included in the tumor. In the fully developed deciduomata the structure of the tumor resembles very much that of the ordinary decidua of the placenta of the guinea-pig.

3. After the deciduomata have reached their full growth the cells very rapidly die, and the tumor becomes necrotic. Thirteen days after the operation was the latest period at which a few mitoses could still be seen. In one case, as late as seventeen days after the operation, the deciduomata were still alive, but no mitoses were present in these tumors; in the latter two cases the animals were pregnant. As a rule, fifteen days after the operation the deciduomata have become entirely necrotic, and they gradually shrink.

4. I thought it possible that co-existing pregnancy might prolong the life of the deciduomata. In a series of experiments, therefore, I incised only one horn of the uterus, with the hope that in the other horn pregnancy might develop. In only three cases did I succeed. In two of these, thirteen and seventeen days after the operation, respectively, the deciduomata were still alive; but in the latter case cell proliferation had ceased. In the third case, twenty-one days after the operation the deciduomata were necrotic, notwithstanding the presence of living embryos in the other uterine horn. Necrosis, therefore, cannot be prevented by the co-existence of pregnancy, although it is possible that the life of the deciduomata may be slightly prolonged under these conditions.

5. These deciduomata cannot be produced in the uterus of every female guinea-pig. In cases in which they are not obtained the mucosa of the uterus shows merely an edematous, hyperemic condition, without cell proliferation. They can be produced (*a*) at certain periods after copulation. In eight cases in which the operation was done inside of twenty-four hours after copulation no deciduomata were formed. In only one case in which the operation was performed eighteen hours after copulation was a trace of decidual tissue detected on microscopic examination. Nine animals were operated upon on the second day after copulation. In these animals, either no deciduomata developed, or those that did develop were very small. In one case the operation was performed three days after copulation; and small deciduomata were found after ten days. Of twelve animals operated upon four days after copulation the deciduomata developed in ten.

In one case in which the animal was examined twenty days after operation no deciduomata were found. Possibly, however, a small deciduoma may have been dissolved at a period long after operation, so that this case cannot be fully counted as negative. In the second negative case, the animal had been dead a long time before autopsy. This case is probably to be regarded as negative.

Twenty-three guinea-pigs were operated upon five days after copulation; all of them developed deciduomata. Among eight guinea-pigs operated on six days after copulation, seven developed deciduomata; one case was negative. In the latter case the autopsy was made twenty-one days after operation, and the animal had had young

ones shortly before the operation. It is possible that in this case also the deciduoma may have disappeared. Thirteen guinea-pigs were operated on seven days after copulation. Twelve of these developed deciduomata. One case was, perhaps, negative. Microscopic examination is still to be made in this case. This negative result may be explained through the special kind of incision used in this instance. Two guinea-pigs, operated on eight days after copulation, both developed deciduomata. Four guinea-pigs were operated on nine days and ten days after copulation, respectively. In one case in which the operation was done not quite nine days after copulation, pregnancy developed and simultaneously small decidual nodules were found. Two other cases were negative; and in the fourth case deciduomata developed. In five cases, cuts were made into the uterus in more advanced stages of pregnancy, but in none of these did deciduomata develop.

SUMMARY. From the fourth to the eighth day after copulation deciduomata almost invariably develop if the correct incisions have been made. Inside the first day after copulation no deciduomata usually develop. On the second day only very small deciduomata develop, or none at all. On the ninth and tenth days after copulation, again, the formation of deciduomata becomes very uncertain, and it is absent in the later stages of pregnancy. There exists, therefore, a definite curve that indicates the chances for the development of deciduomata after copulation. The optimum lies between five and eight days thereafter, and this corresponds with the time when the uterus normally responds to the stimulation of the ovum with the formation of a decidua.

6. We see, therefore, that (*a*) at certain stages after copulation the uterus is able to form a very large number of deciduomata if it is stimulated in the proper way. A preceding copulation is not, however, necessary for the formation of deciduomata. (*b*) At certain periods after the condition of heat has passed in animals their uterus likewise responds to the proper stimulation with the production of deciduomata.

Fifteen animals were operated upon inside of the next ten days after the period of heat. In twelve of these cases deciduomata developed. In two of these cases the deciduomata were very small, namely, in the

cases in which the animals were operated upon very soon after the period of heat (twenty-one to thirty-six hours, respectively). In the other positive cases the animals were operated upon four to six days after the period of heat. In the three other cases no deciduomata developed after the operation. In one of these, the operation was done two days after the period of heat; in another, three days thereafter; and in the third, five days afterward.

We know that even after copulation the experiments performed two days thereafter are not always successful; and in the other two negative cases, namely, three and five days after the period of heat had passed, respectively, a special method of incision was used. This incision was also used in a few other cases, and did not yield satisfactory results. In six of these fifteen cases the ovaries were examined microscopically in serial sections, and corpora lutea were found to be present.

7. We found that at a later period than ten days after copulation deciduomata did not develop. Correspondingly, we find that if we operate thirteen to sixteen days after the period of heat, deciduomata almost invariably do not develop. Eighteen animals were operated upon at this period. Seventeen did not develop deciduomata. In only one case did the deciduomata develop. It is, of course, not impossible that in this case either a mistake may have been made in determining the period of heat or some complicating factor was added. In four of these eighteen cases the ovaries of which were examined microscopically in serial sections, no corpora lutea were present.

8. The period at which no deciduomata were found is followed by another period in which the uterus again becomes responsive. In four cases guinea-pigs were operated upon eighteen to twenty-one days after the period of heat without preceding copulation. In one case no deciduomata developed, and no corpus luteum was present in the ovary. In another case no deciduomata could be seen macroscopically. Whether microscopically decidual tissue was present has still to be determined. In the third case, in which a corpus luteum was present, many deciduomata were formed, and in the fourth no real deciduoma was produced. There was, however, a spindle-cell growth present beneath the epithelium, with mitoses in the spindle-cells—a pre-decidual condition.

9. *Summary:* We see, therefore, that animals, at certain stages of their sexual life, can form deciduomata without a preceding copulation. Here, also, we notice the presence of a certain curve, the optimum of which lies approximately at four to nine days after the period of heat. From thirteen to sixteen days the chances for decidual formation are almost nil; and then gradually the uterus seems again to become responsive.

10. The ovaries of six guinea-pigs that had been operated upon inside of ten days after the period of heat were examined microscopically in serial sections. They all showed the presence of corpora lutea. In all these animals deciduomata developed. The ovaries of five of the guinea-pigs that had been operated upon thirteen to sixteen days after the period of heat were likewise examined in serial sections microscopically. In four cases no corpora lutea were found. In one case the corpora lutea had been present. At this time the deciduomata, as I stated above, do not develop. The ovaries of three guinea-pigs that, according to the statement of the breeder, had been in the period of heat inside of the last ten days (but that did not, after operation, develop deciduomata), did not, on microscopic examination, show the presence of any corpora lutea. Four guinea-pigs that had apparently not been in heat for some time were operated upon in the usual way. Only one of these developed deciduomata. Eight guinea-pigs in which the period of heat had not been determined previously were taken at random and operated upon in the usual way. In these cases, of course, it is possible that a certain number of the majority of the animals may have been in a favorable condition for the development of deciduomata, but after operation, in seven, the uterus healed without the formation of deciduomata, and only in one of them did deciduomata develop.

11. These differences between the results obtained after operation upon animals from the third to the ninth day after the period of heat, on the one hand, and those obtained with guinea-pigs operated upon at other periods, on the other hand, are so striking that we may conclude that the development of the deciduomata depends upon a certain condition in the sexual cycle of the animal at the time of operation.

12. In order to obtain a deeper insight into the causes that lead to this peculiar predisposition to the development of deciduomata, several

series of experiments were made. It was possible that the contact of the ovum with the mucous membrane might cause the predisposition of the mucous membrane to the development of the deciduomata. This, however, is very improbable, because deciduomata can develop in horns of the uterus without the corresponding ovaries containing a corpus luteum. It is, furthermore, improbable, because the deciduomata can develop below the seats of pregnancy. I could exclude this hypothesis with certainty in the following way:

One or two days after copulation, before the ovum had passed into the lower part of the uterus, a ligature was applied to the centre of both horns. This prevented the ovum from touching the mucous membrane of the lower half of the uterus. A few days later the uterus below and above the ligature was incised in the usual way, and in three cases treated in this manner deciduomata developed both above the ligature and below it. We may, therefore, state that the contact of the ovum with the mucous membrane is not necessary for the development of the deciduomata.

13. The second series of experiments that we made in order to clear up the peculiar predisposition of the uterus in certain animals consisted in the extirpation of the ovaries, before the incisions into the uterus were made in guinea-pigs that had previously been copulated. Twenty-nine guinea-pigs were operated upon in this way. In twenty-one of them the incisions into the uterus were made from two to five days after the extirpation of the ovaries. In seventeen of these no deciduomata developed. In one animal there developed only microscopically recognizable deciduomata. Two died prematurely, and one developed an abscess. Of eighteen guinea-pigs, therefore, seventeen were without deciduomata, and one had only a microscopically recognizable deciduoma, although all of them had been operated upon at the most favorable time after copulation. In eight guinea-pigs, incisions were made into the uterus immediately after the ovaries had been extirpated, or one day later. In three of them, at most places of incision no deciduomata developed, and only at one or two places small microscopic deciduomata were found. In one, a somewhat larger microscopic deciduoma developed; the others were entirely free from deciduomata. From these experiments we may conclude that extirpation of the ovaries in most cases prevents entirely

the formation of deciduomata in animals that are operated upon at the most favorable period after copulation.

14. In accordance with the results of these experiments it was found that of five guinea-pigs that had been taken from the common stock without any knowledge of their period of heat, and whose ovaries had been extirpated, after a subsequent incision of the uterus, four did not develop deciduomata. In one case small microscopic deciduomata were found.

15. In order to rule out the objection that a previous operation had in itself prevented the formation of deciduomata in three guinea-pigs, another series of experiments were carried out, in which the ovaries were merely pulled out of the wound without being extirpated. In all cases a second operation was followed by the formation of deciduomata.

16. In eight other guinea-pigs that had been previously copulated the ovaries were cauterized at various places several days before incising the uterus. Six of these developed deciduomata. One died prematurely, and in one case a microscopic examination has still to determine the outcome. We see, therefore, that a previous ovarian operation does not in itself interfere with the formation of the deciduomata, if after the first operation the uterus is later incised in the usual way during a second operation.

17. These experiments establish the fact that the presence of the ovaries is responsible for the peculiar predisposition of the uterus at certain periods, which enables it to form deciduomata under the influence of certain indifferent stimuli. The question next to be determined was, whether the influence of the ovaries is transmitted through nerves, or whether we have to deal with the effect of chemical substances secreted by the ovaries. In order to determine this problem, I transplanted pieces of the uterus of guinea-pigs into the subcutaneous tissue at the proper period after copulation. It was found that in two cases deciduomata developed in pieces of uterus transplanted into the subcutaneous tissue. This makes it probable¹ that

¹ It is very unlikely that an ovum had been transferred with the piece of uterus. No embryo could be seen in the transplanted pieces. It is, likewise, very improbable that such a proliferation would take place in guinea-pigs under any conditions irrespective of the state of the ovaries. Further experiments will decide these points with certainty.

an "internal secretion" of the ovaries is responsible for the above mentioned predisposition of the uterus to form deciduomata.

18. Certain facts that have been mentioned before suggest that the corpora lutea, which are present in the ovaries after copulation, and probably at certain times after the period of heat, represent that part of the ovary that secretes the substance necessary for the development of the deciduomata. In nineteen guinea-pigs we accordingly attempted to burn out the corpora lutea soon after copulation, and at a second operation the usual incisions into the uterus were made. One of these nineteen animals died too early to be used. Of the others, four developed good deciduomata; but the microscopic examination of the ovaries showed that not all the corpora lutea had been destroyed. In each of these cases corpora lutea had been left in the ovaries. In four other cases in which deciduomata were produced a complete examination of the ovaries has not yet been made. Four other guinea-pigs did not develop deciduomata. Microscopic examination of their ovaries showed the absence of corpora lutea. In one of these four cases one ovary only has so far been examined. In two animals, in which only small microscopic deciduomatous areas developed, the corpora lutea were entirely or almost entirely destroyed. In four other cases no deciduomata developed, but the ovaries have not as yet been examined.

19. In seven guinea-pigs only one ovary was removed. Five of them developed deciduomata. In two of these cases the ovaries were examined microscopically, and corpora lutea were found in the remaining ovary. In one no deciduoma developed, and the examination showed that no corpus luteum was present in the remaining ovary. In one case, only small doubtful nodules were found without a microscopic examination of the uterus or ovaries having as yet been made.

20. All these facts show that a certain substance secreted by the ovaries is responsible for the formation of the deciduomata, and make it at least very probable that the corpora lutea represent that part of the ovary which secretes this substance. This latter point, however, can only be made certain by further experiments, which are under way at the present time.

21. A certain substance present in the ovaries is necessary for the

production of deciduomata. If, as we found, very small deciduomata can be formed, even after extirpation of the ovaries, it is most likely that a certain amount of this substance is still present in the circulation at the time of the incision into the uterus. We noticed especially the fact that extirpation of the ovaries is followed by the formation of microscopic deciduomata in such cases, in which the uterine operation was carried out very soon after the extirpation of the ovaries, and this circumstance speaks in favor of the interpretation just given.

22. The process leading to the formation of the deciduomata differs in essential points from the connective-tissue proliferation in ordinary wound healing: (a) The production of new cells by mitotic division is incomparably more extensive in the development of deciduomata. For some time the proliferative energy resembles closely the growth of a malignant sarcoma. (b) Instead of forming mainly connective-tissue fibrils, the new cells enlarge and assume more or less the appearance of decidua cells. Scar tissue is not produced. (c) Instead of growing into the wounds and filling the defects, the cells grow upward between the glands of the uterine mucosa and raise the epithelial covering of the uterine mucosa. (d) At a certain stage of development the whole new formation becomes necrotic.

23. As we have stated above, the deciduomata become invariably entirely necrotic twelve to twenty days after operation. This might be due to interference with the circulation. In cases of rapid cell proliferation, the development of bloodvessels frequently does not proceed adequately, and we find, therefore, often, in rapidly growing tumors, areas of necrosis. Such an interpretation can, however, not explain the invariable necrosis of the deciduomata at a certain period of their development. Such a necrosis takes place quite independently of the condition under which they develop. It is always complete, even if at certain places the bloodvessel supply is quite sufficient. This necrosis must be compared to the complete disappearance of the corpus luteum, after the causative factors leading to its development have ceased to exist. We saw that certain substances prepare the uterine connective-tissue cells to proliferate and to form a deciduoma. It seems, therefore, evident that the same substances which prepared the cells for the proliferation are necessary for their life. The cells die as soon as the "growth substances" have disappeared.

24. A problem of great interest, which at the present we merely wish to state as such, is the limitation of the proliferation to the connective tissue of the uterus. The subserous or subcutaneous connective tissue, which is likewise incised, does not form nodules. Does that depend on a physicochemical difference of connective-tissue cells in different parts of the body, or does it depend on the specific relation of connective-tissue cells to certain differentiated cells in their neighborhood, in this case upon the influence exerted by the uterine epithelium? The latter interpretation seems to be the more probable one.

25. If, as we saw, a certain "internal secretion" of the ovaries is of great significance for the production of deciduomata, we are justified in concluding that it has the same significance for the formation of the placental decidua. The early extirpation of the ovaries, and not unlikely the early destruction of the corpora lutea, interferes with the normal course of pregnancy, because the substance which is necessary for the development of the decidua is absent. These experiments will, therefore, decide the validity of the Born and Fraenkel hypothesis in regard to the significance of the corpus luteum for the development of pregnancy, and they will, furthermore, define the character of this influence exerted by the corpus luteum.

26. Toward the end of the first week following copulation the ovum implants itself into the uterine wall and causes the decidua formation which is necessary for embryonic development. We have here an instance of an especial adaptation. The uterus responds at the proper time in the proper way in order to insure the development of the embryo. Our experiments permit us a further analysis of this adaptive process. We saw first that an ovum is not necessary for the stimulation of the uterine mucosa, that we have to deal with a two-fold cause; secondly, that the uterus has the potential power to produce many more placentas than it can ever be called upon to do under normal conditions; third, that the stimulus of the ovum alone would probably be powerless without the secretion of the predisposing substance by the ovary. It is especially noticeable that the action of this "preparing" substance becomes potent exactly at that time, when the ovum is ready for implantation; but, as we saw, even without copulation this substance is secreted and acts upon the uterus.

27. In conclusion, I wish to point out a certain analogy which exists between these artificially produced deciduomata and a variety of multiple tumors that are limited to certain organs, as, for instance, multiple fibroneuromata, enchondromata, symmetric lipomata, or adenomata of the intestinal mucosa. All of these might be called multiple systemic tumors. The deciduomata represent a type of new formations which I designated "transitory tumors." If the substance were secreted by the ovaries continuously instead of intermittently, the tumors would probably lose their transitory character and would become permanent newgrowths. In the case of the systemic tumors and of the deciduomata we have to deal with multiple, more or less benign tumors affecting one organ or one tissue. We know that the origin of the deciduomata depends upon two sets of conditions: (a) a predisposing chemical substance is produced by a certain organ, and (b) if such a substance has been produced, indifferent stimuli, for instance traumatisms, are sufficient to produce the tumors. Clinical observation makes it likely that certain tumors, as, for instance, sarcomata, have at times been caused by a traumatism. Experimentally, attempts to produce tumors through traumatism, or through long-continued irritation, have never been successful. It may be suggested that such attempts could only have been successful if the necessary "preparatory" substance had been secreted prior to the action of the indifferent stimuli.

As stated above, the new formations which we produced differ in several essential aspects from regenerative proliferation of the connective tissue during wound healing. Furthermore, the processes leading to the formation of deciduomata have nothing in common with so-called inflammatory reactions. Neither do they represent an example of compensatory hypertrophy, although there exist cases of compensatory hypertrophy which, apparently, almost imperceptibly, merge into adenomatous new formations, just as certain hypertrophic processes taking place in the connective tissue in connection with wound healing lead to the production of keloids, which are usually classed among the fibrous tumors. If we injure the perichondrium near the ear cartilage the regenerative processes setting in may lead to the formation of small nests of cartilage. A similar result can be obtained if we transplant pieces of perichondrium. It is very doubtful

whether we should call such small cartilaginous areas "chondromata." But let us assume that under the influence of an abnormal internal secretion, which suddenly comes into play, the connective tissue of the mucosa of the intestines began to proliferate at circumscribed areas in the neighborhood of places of injury, raising up the epithelium and forming relatively large polypoid outgrowths covered by epithelium, we would be much tempted to regard such multiple polypoid outgrowths as related to tumor formation, especially if the connective tissue of the mucosa showed a metaplasia into another variety of connective tissue. A sharp line of demarcation between regenerative and tumorous new formations does not exist. We are inclined to use the term "tumor" if the cell proliferation is very marked and limited to a certain circumscribed area, and leads to a well-defined new formation; and if, and this is an essential point, we cannot entirely account for the tissue proliferation by including it among the regenerative or inflammatory reactions, if there exists an unknown factor leading to the marked cell growth.

In the case of the deciduoma we have found the definite cause for its formation, and it is very desirable to emphasize certain similarities between the deciduoma and various other tumors in order to indicate the possible presence of predisposing "preparing" substances as the unknown cause of certain tumors. Given the presence of such a "preparing" substance, otherwise indifferent stimuli would be sufficient to excite the potential proliferative energy of the tissues. The fact that the deciduomata degenerate as soon as the "preparing" substance ceases to be active is no valid reason for denying the designation "tumor" to these new formations. In order to indicate the ephemeral character of such new formations, they may be called "transitory tumors." Even carcinomata may retrogress spontaneously. As I have pointed out in another paper,¹ the presence of a "preparing substance can only explain the formation of a "transitory tumor," or, at the best, of a tumor that grows indefinitely in the same individual in which it originated, but it cannot explain the growth of a tumor which can be transplanted into many other individuals in which such a "growth" substance is not likely to be present. In order to

¹ Über einige Probleme der exper. Tumorforschung. Zeitschrift f. Krebsforschung, 1907, Band v, Heft 3, pp. 17, 18.

explain on such a basis the inoculability of tumors, we would have to assume the hereditary transmission of an increased energy of growth to the following generations of tumor cells, which thus would be able to continue to proliferate without the further presence of the growth substance in the inoculated animal. The possibility of such a transmission into later generations has not yet been established. Until such a proof has been given we must assume that transplantable tumors carry with them in the tumor cells or in their direct neighborhood the stimulus which enables them to proliferate in a new host. But it is quite possible that a non-transplantable tumor which originated through the action of a "preparing" substance may grow very rapidly, and be, therefore, malignant. Transplantable tumors, on the other hand, do not need to be very malignant. As I have pointed out,¹ the degree of inoculability and energy of tumor growth are two distinct properties which do not need to be associated in the same tumor. But the transplantability of tumors depends, in all probability, not only on the presence of a permanent stimulus in or near the tumor cells, but on some other factors as yet unknown. The presence or absence of such secondary factors might determine the inoculability or non-inoculability of a tumor, even if the essential cause in the tumor formation was the same in both cases. *April 9, 1908.*

The Colon-aërogenes Group of Bacteria.

By D. H. BERGEY, M.D., AND SYLVESTER J. DEEHAN, M.D.

THERE still exists among bacteriologists considerable confusion and uncertainty with regard to the identity of bacteria capable of fermenting carbohydrates, encountered in feces, in water, in milk, or elsewhere.

During the past two or three years this group of bacteria has been studied in detail by a number of competent observers in England, Germany, and in America, and we are today in a better position to identify these carbohydrate fermenting bacteria than we have

¹ Ueber einige Probleme der exper. Tumorforschung. *Zeitschrift f. Krebsforschung*, 1907, Band v, Heft 3, pp. 18, 19.

ever been before. The important investigations that have been of value in differentiating this group of bacteria are those of MacConkey (*Journal of Hygiene*, vol. v, p. 333; vol. vi, p. 385), those of Twort (*Centralblatt f. Bakteriologie*, Referate, Band xl, p. 508) and Vourloud (*Centralblatt f. Bakteriologie*, 1 Abt. Originale, Band xlv, p. 97), and those by Winslow and Walker (*Science*, December 6, 1907, N. S., vol. xxvi).

The studies of these investigators have shown that the bacteria belonging to the colon-aërogenes group may be differentiated from each other by their fermenting powers when tested upon various carbohydrates. Besides these tests, MacConkey has shown that several other factors are of service in differentiating the members of this group of bacteria, namely, the presence or absence of liquefaction of gelatin, the presence or absence of indol production, the presence or absence of motility, and the presence or absence of the Voges and Proskauer reaction (*Zeitschrift f. Hygiene*, 1898, vol. xxviii, p. 20).

After testing the fermenting powers of the bacteria of this group upon quite a large number of different carbohydrates, MacConkey found that the carbohydrates which were of greatest importance in the differentiation of the different members of this group of bacteria were saccharose, dulcitol, adonitol, and inulin. MacConkey tested the fermenting powers of these bacteria in the ordinary nutrient media, containing the different carbohydrates, as well as in bile-salt media colored with neutral red. Twort used the usual peptone solution, to which had been added the carbohydrates in the proportion of 2 per cent. Vourloud employed the ordinary 2 per cent. agar, to which the carbohydrates had been added, using litmus as his indicator. In our own work we have employed the Hiss serum-water media colored with litmus, to which the different carbohydrates were added in the proportion of 1 per cent.

There has been so much uncertainty with regard to the biological characters of this group of bacteria that a number of them have been described under several different names. The better-known organisms of this group are *Bacillus coli communior* and *Bacillus coli verus*, two types of colon bacillus, both of which ferment dulcitol, the former also fermenting saccharose. *Bacterium aërogenes* (Escherich), *Bacterium acidilactici* (Hüppe), *Bacterium pneumoniae* (Friedlander),

Bacillus cloacæ (Jordan), *Bacillus enteritidis* (Gaertner), *Bacillus vulgaris*, *Bacillus icteroides* (Sanarelli), *Bacterium neapolitanus*. To these organisms MacConkey has added *Bacillus Grünthal*, an organism found by Fischer in cases of meat poisoning (*Zeitschrift f. Hygiene*, 1902, Band xxxix), *Bacillus levans*, an organism which Wolffin Holiger (*Centralblatt f. Bakteriologie*, Abt. II, Band ix, p. 305) found to be the cause of fermentation of dough; *Bacterium coscoroba* (*Annales de l'Institut Pasteur*, 1900), which was found to be the cause of an epidemic in swans; and *Bacterium oxytocus* (Wyssokowitsch), an organism which had been isolated from stale milk.

MacConkey has found that *Bacillus cavidica* (Brieger) and *Bacillus mustilæ septicus* are identical with *Bacillus coli verus*. He also found that *Bacterium rhinoscleromatis* and *Bacterium ozenæ* are likewise identical with *Bacterium pneumoniæ* (Friedlander), while *Bacterium aërogenes* and *Bacterium capsulatus* (Pfeiffer) are also identical.

The value of the Voges and Proskauer reaction has been called in question by Harris (*Bulletin de l'Institut Pasteur*, 1906, vol. iv, p. 250), but MacConkey contends that the reaction is reliable, and our experiments confirm entirely this contention of MacConkey's. In making the test it is important that the culture medium employed be not highly colored with beef extract, otherwise there may be some uncertainty as to the presence or absence of this color reaction. The reaction in question is a change in color taking place in the fluid of the fermentation tube, after absorption of the carbon dioxide by means of sodium hydroxide. The presence of the reaction is indicated by the change of the color of the fluid to one closely resembling eosin, the substance present being acetylmethylcarbinol (Harden, *Proceedings of the Royal Society*, 1906, vol. lxxvii, p. 424). This substance seems to be produced by some bacteria that decompose dextrose. The characteristic color when this substance is present begins to show itself about twenty-four hours after the sodium hydroxide is added, and is usually most intense after forty-eight hours.

The value of these different tests in the differentiation of the bacteria of this group is dependent directly upon the stability of the different cultural characteristics of bacteria. If it is possible by any means whatever to make bacteria acquire properties which they do not possess ordinarily, then the value of these tests becomes practi-

cally *nil*. MacConkey is emphatic in his statements that it is impossible to make bacteria take on new characteristics, even by long-continued cultivation. For instance, if an organism is capable of fermenting only one of the four carbohydrates which he has found serviceable for purposes of differentiation, such organism will always ferment this carbohydrate, and none of the others. Twort (*loc. cit.*) claims to have been able to bring about the fermentation of carbohydrates by bacteria that ordinarily do not possess this power. In our own experiments we have failed entirely to arouse fermentative powers in an organism that did not originally possess these powers, and, in the same way, all our attempts to induce bacteria to alter their biological character with regard to the liquefaction of gelatin, and the production of the Voges and Proskauer reaction, have failed completely. In this respect we are fully in accord with MacConkey's contention, and are unable in any particular to uphold the statements of Twort. Our position is in accordance with the accepted views on heredity, since all of the more important of the scientists of the present day claim that acquired characteristics are not transmissible.

It is well known to what a marked degree cultures of known organisms may become altered as to their morphological, biological, and pathogenic properties, but careful investigations into the nature of these alterations show that they are degenerative in character and not evolutionary. When, by any means, we increase the virulence of an organism, we merely restore to it in full degree its normal properties—properties that have been lowered through the unfavorable influences of environmental conditions.

The studies embodied in this report were made upon the bacteria isolated from fifty samples of milk, from one sample of kefir, and from ten samples of sewage. The sewage organisms were isolated and studied by Dr. Deehan, who is entitled to equal credit for the results embodied in this study. The organisms obtained from milk were isolated and studied by Dr. Bergey. Several of the samples of milk examined were samples of "certified" milk, but none of the organisms included in this study were found in this milk. All the other samples of milk were ordinary market milk, derived from milk shops in different parts of the city. One of the organisms was isolated from a sample of kefir.

Only such organisms have been included in the present study as are closely allied to either *Bacillus coli* or *Bacterium aërogenes* in general cultural characters, and in the possession of the power of fermenting dextrose.

We have grouped the bacteria of the colon-aërogenes group according to their powers of fermenting saccharose, dulcitol, adonitol, and inulin, and with regard to the presence or absence of motility, indol production, the Voges and Proskauer reaction, and liquefaction of gelatin. Taking the latter four characters, we may have sixteen different possibilities as to the presence or absence of combinations of these reactions. In the same way, taking the power of fermenting the four different carbohydrates, we may also have sixteen different possibilities with regard to these powers. We have conceived, therefore, that it might be possible to have 256 different combinations of these eight reactions. We have arranged the bacteria of this group, therefore, into sixteen sub-groups, which we have numbered from *a* to *p*.

In a general way, the system of classification followed here is that proposed by MacConkey. We have rearranged the location of the different factors in the charts and have greatly extended the number of possible combinations that may occur.

MacConkey has included in his chart the percentage of gas formed in 1 per cent. dextrose bouillon, and the relation of the proportion of hydrogen gas formed to the amount of carbon dioxide formed. While we have found these factors of some value, they are, however, of less importance than others on which we have laid greater stress.

While it is evident that this method of classification is of great value, it fails to allow us to differentiate some organisms that have been regarded as distinct species. Extended study along these and other lines must eventually prove or disprove the value of the method followed.

The organisms in group *a*, comprising the first sixteen of the 256 possibilities, are those which ferment none of the four carbohydrates. In this group the only known organism is *Bacillus Grünthal*. We have found in the organisms isolated from milk four other representatives of group *a*. Group *b* comprises the organisms that ferment saccharose, but none of the other three carbohydrates. The known

organisms of this group are *Bacterium coscoroba*, *Bacillus vulgaris*, and *Bacillus cloacæ*. Besides these, we have found four other representatives of this group in milk. Group *c* comprises the organisms which ferment dulcitol, but none of the other carbohydrates. The known organisms in this group are *Bacillus enteritidis* and *Bacillus coli verus*. Besides these, we have found two other representatives of the group, while MacConkey has found one of these unnamed representatives a number of times, and, besides this, he has found one other representative of this group which we did not encounter. Group *d* comprises the organisms which ferment adonit, but none of the other carbohydrates. The known organisms of this group are *Bacillus icteroides* and *Bacterium acidi lactici*. We have not found any other representative of this group, but MacConkey has found an additional organism. Group *e* comprises the organisms which ferment inulin, but none of the other carbohydrates. The only known organism of this group is *Bacillus levans*. This organism was not found in milk, but was isolated from sewage. No other organisms belonging to this group were encountered. Group *f* comprises the organisms which ferment both saccharose and dulcitol, but neither of the other carbohydrates. The known organisms of this group are *Bacterium neapolitanus* and *Bacillus coli communior*; aside from these, we have found one other representative of the group. Group *g* comprises those organisms capable of fermenting saccharose and adonit, but neither of the other sugars. The only known organism found in this group is *Bacterium aërogenes*. Aside from this, we have encountered two other species, which had also been found by MacConkey, and, in addition to these, MacConkey found another species. Group *h* comprises those organisms capable of fermenting saccharose and inulin, but neither of the other carbohydrates. There are no known organisms belonging in this group, and we have found only one organism representing this group. Group *i* represents the organisms capable of fermenting dulcitol and adonit. Group *j*, those organisms capable of fermenting dulcitol and inulin. Group *k*, those capable of fermenting adonit and inulin. There are no known representatives of these three groups, and we have found no bacteria belonging to these groups. Group *l* comprises the organisms capable of fermenting saccharose, dulcitol, and adonit, but do not attack inulin. The only

known organism of this group is *Bacterium pneumonicum*. Aside from this organism, MacConkey has found two other representatives of the group, while we have encountered two additional representatives. Group *m* comprises the organisms capable of fermenting saccharose, dulcit, and inulin, but do not attack adonit. There are no known representatives of this group, but we have encountered one organism belonging to this group. Group *n* comprises the organisms capable of fermenting saccharose, adonit, and inulin, but do not attack dulcit. There are no known representatives of this group. MacConkey has found two organisms belonging in this group, one of which was also encountered by us, and, in addition to this, we have encountered one other representative of the group. Group *o* comprises the organisms capable of fermenting dulcit, adonit, and inulin, but do not attack saccharose. There are no known representatives of this group, and we have encountered none. Group *p* represents those organisms capable of fermenting all of the four sugars. The only representative of this group is *Bacterium oxytocus*; besides this, MacConkey found another representative of this group, an organism which we also encountered, and, in addition to this, we encountered a third representative of this group.

The organisms which we isolated from milk represent twenty-seven different species, only five of which are known species, and seven of the other species had been encountered before by MacConkey, while the other fifteen species appear to be new.

The organisms which we isolated from sewage represent nine different species, all of which had been previously described.

The seven organisms isolated from milk which MacConkey has also found, but not named, correspond to the following numbers in our system of classification: 3, 35, 99, 101, 213, 215, and 247.

The fifteen organisms isolated from milk which appear to be unnamed, and which were not encountered by MacConkey, correspond to the following numbers in our system of classification: 2, 6, 8, 18, 20, 22, 26, 38, 86, 116, 177, 183, 198, 211, and 248.

No doubt most, if not all, of the undescribed bacteria encountered had been found by other investigators, but indefinite descriptions prevent us from being absolutely certain of it.

All bacteriologists have frequently encountered bacteria which

resembled *Bacillus coli* in many respects, but differed from it in one or more particulars, and it has always been a matter of regret, personally, that it was impossible to give more definite classification of such organisms than to say that they belong in the "colon" group.

The studies of MacConkey and others indicate that all of the bacteria of this group are more or less constant and normal inhabitants of the intestines of man and the domestic animals, and that the discovery of these organisms in milk, water, or other food products is an indication of fecal contamination. There is as yet no means of determining the remoteness of the contamination, neither is it possible to determine whether the contamination is of human or animal origin.

March 26, 1908.

Acute Lymphopenic Lymphatic Leukemia.

By R. S. LAVENSON, M.D.

THE case forming the substance of this report presented a group of interesting and unusual features. Some of them would be considered as quite out of harmony with the generally accepted view of the nature of leukemia. I think, however, that an analysis of the case will establish its identity as one of the more unusual forms of this disease.

The patient, A. D., was a dentist, aged forty years, who had been in practice for the past five years. Previous to this time he had lived on a ranch in the southern part of the United States. He was admitted to the University Hospital on January 1, 1907, under the care of Dr. Frazier, to whom I am indebted for permission to use these clinical notes.

Family History. Aside from the fact that several maternal uncles had died of tuberculosis, nothing in the patient's family history had any bearing upon his present condition.

Personal History. The patient had always been a man of good habits. He used neither alcohol nor tobacco to excess. He had been married for ten years. His wife had had no children and no miscarriages. The patient denied venereal infection.

Previous Medical History. With the exception of the diseases of childhood, the patient had always enjoyed good health.

History of Present Illness. In the beginning of November, 1906, after having eaten rather liberally of pineapple, which he thought irritated his mouth, the patient developed an ulcer on the hard palate. This grew slowly but progressively worse, and about a month after its commencement he noted an ulceration of the gums of the lower jaw, localized to the lingual surface of the right side and the buccal surface of the left side. At about the same time the submaxillary lymphatic nodes became slightly enlarged and painful, first on the right side, and later on the left. The severity of these lesions steadily increased, but the patient was able to attend to his professional duties until a few days before his admission to the hospital.

Condition on Admission. On admission to the hospital the patient's temperature was 102°; pulse, 84; respirations, 24. His only complaints aside from feeling slightly feverish were soreness of the mouth and some difficulty in swallowing. He was found to be a man of good development and nourishment, and powerful physique. The physical examination, aside from that of the mouth and the adjacent lymph nodes, presents absolutely no features worthy of mention.

Mouth. The hard palate from the incisor teeth to within an inch of the uvula, the lingual surface of the right lower gingives, and the buccal surface of the left lower gingives are the seat of an extensive ulceration. The edges of the ulcerated areas are irregular but not ragged, nor have they a punched-out appearance. They do not bleed readily on being touched, and they present no evidences of granulations. The central portions of the ulcerations consist of a grayish-white slough, rather firmly adherent to the underlying tissues. The bone is apparently not involved. The entire ulcerated area has an extremely offensive odor. The tongue is covered with a heavy, shiny, grayish-yellow coating. The teeth are in good condition.

The submaxillary lymph nodes on the right side are slightly enlarged, firm, and tender; those on the left side are smaller and less tender.

Urine. Light amber; acid; specific gravity, 1022; no sugar or albumin; a moderate amount of mucus; numerous leukocytes, epithelial cells, and calcium oxalate crystals.

Sputum. Seropurulent; yellowish white; offensive odor; numerous leukocytes and epithelial cells; a few erythrocytes.

Bacteriological Examination. Exhaustive bacteriological studies by Dr. D. H. Bergey revealed the following findings: In smears from the ulcerated areas there were found in great numbers streptococci, spirochetes, and an organism resembling the *Bacterium fusiformum*. Aërobic cultures made from the same material showed practically a pure culture of streptococcus. Anaërobic cultures revealed streptococci and the *Bacterium fusiformum*. Sections taken from the diseased area and stained for organisms showed streptococci and a rod-shaped organism, apparently the *Bacterium fusiformum*. With specific stains no spirochetes or tubercle bacilli were found.

Blood Examination. Hemoglobin, 75 per cent.; erythrocytes, 3,400,000; leukocytes, 5400. Differential count: polymorphonuclear neutrophils, 75 per cent.; lymphocytes, 19 per cent.; large mononuclear leukocytes, 4 per cent.; transitional cells, 2 per cent.; eosinophiles, none.

With these facts at hand, the etiology of the condition was extremely obscure, and a diagnosis was not made. Despite the patient's denial of venereal infection, antisyphilitic treatment was instituted on the possibility of the ulceration being specific.

The subsequent course of the disease will be given as briefly as possible, consistent with a proper understanding of the case.

January 10. Slight swelling and tenderness of the axillary lymph nodes on both sides was noted today. Temperature range has been between 100° and 103°. Patient has been uncomfortable and restless. Ulceration is slowly spreading. Leukocytes, 6800.

15th. Blood culture reported sterile. Injections of diphtheria antitoxin instituted.

20th. Patient has been receiving 9000 units of diphtheria antitoxin daily. Temperature has been showing a gradual downward tendency. The ulceration in the roof of the mouth is showing distinct signs of improvement; in the posterior portion granulations have appeared and the odor has decidedly decreased.

25th. Granulations on the hard palate progressing. The ulcerations on the lower gums increasing slightly. Temperature decreasing. Leukocytes, 6300. Diphtheria antitoxin discontinued.

February 2. Injections of diphtheria antitoxin renewed. Leukocytes, 4500.

10th. General condition considerably improved. Ulcerations continue to show slight improvement. Patient discharged to go to Atlantic City, where antitoxin injections will be continued under the supervision of Dr. Emery Marvel.

20th. Patient readmitted. On frontal bone, just above right eye, is a tumor the size of a robin's egg. It is rather soft, but not fluctuating, and is not movable. General condition and condition of mouth about the same as on departure for Atlantic City.

Blood Examination. Hemoglobin, 72 per cent.; erythrocytes, 5,210,000; leukocytes, 8000. Differential count: polymorphonuclear neutrophils, 64 per cent.; lymphocytes, 16 per cent.; large mononuclear leukocytes, 12 per cent.; transitional cells, 8 per cent.; eosinophiles, none.

25th. Mass above right eye increasing in size. Right submaxillary lymph nodes slightly larger and more painful. Blood culture reported sterile. A dark-red, umbilicated papule noted on the back of the neck. Lower down on the back are several red, shiny, non-umbilicated papules.

March 2. A number of small, red papules similar to those above described have appeared on the chest. Those on the back have become larger, and some of them have become dark red and umbilicated.

Swelling over right eye increasing. Ulceration in mouth extending. Temperature steadily rising. Patient shows some mental hebetude.

Bacteriological examination of the mass over the right eye by Dr. Bergey showed micrococci similar to but not identical with streptococci; the examination of the umbilicated papules was reported sterile. Leukocytes, 4700.

5th. Eruption has become much more marked over thorax, abdomen, and back. It appears first as small, dark-red, glistening papules; within a day these become larger, and by the next day they have gone into the purplish, umbilicated stage.

The glandular masses on either side of the neck have become so large within the past few days that breathing is difficult.

9th. Almost the entire trunk is covered with the eruption in various stages of development, and there are a few lesions on both arms and

both legs. For the past week the temperature has remained between 102° and 105°.

Leukocytes, 5200. Differential count (500 cells): polymorphonuclear neutrophils, 96 per cent.; lymphocytes, 1.6 per cent.; large mononuclear leukocytes, 1.4 per cent.; transitional cells, 1 per cent.

Died at 7 A.M., March 10.

Autopsy. The postmortem findings will be detailed as briefly as possible, only those tissues being described that presented pathological features. The eruption described in the clinical notes presented the same characteristics after death as it had during life. The distribution of the eruption was general and multiform in character, dependent upon the variations in age of the individual lesions. The remainder of the external examination confirmed the presence of the tumor over the right eye and the swelling of the cervical and axillary lymphatic nodes described in the clinical records.

The spleen was greatly hypertrophied in all directions, and weighed 900 grams. It was pinkish gray, soft, and the follicles were prominent. The only noteworthy feature in the gastro-intestinal tract was a firm, non-ulcerated nodule presenting on the mucous surface of the ileum about two feet above the ileocecal valve. In the cortex of the right kidney was a moderately firm, well-marginated, grayish-red, structureless nodule about the size of a walnut. In the left kidney were three similar nodules about the size of peas. In the middle lobe of the right lung there was a firm, well-marginated, dark-red nodule, and in the lower lobe of the same lung were two others of similar size and consistency. Within the mesentery, and not associated with the mesenteric lymph glands, were a number of firm, well-marginated, pinkish-gray nodules, some of which showed slight surface umbilication. The retroperitoneal, mesenteric, mediastinal, and peribronchial lymph nodes were universally enlarged to the size of large beans. They were pinkish gray in color, firm in consistency, and showed no central softening.

Microscopic Examination. The microscopic examination of the above-described lesions showed one primary common feature—a lymphocytic infiltration. In the skin lesions this infiltration was in the subcutaneous tissue, and extended well into the corium. In some of the older lesions the overlying epidermis was necrotic; in others it was lifted from the corium, forming small bulke, from which the fluid

contents had apparently escaped. In the spleen there was marked follicular hypertrophy, with extensive lymphocytic deposit in the pulp. The nodules in the kidney consisted of dense lymphocytic deposits, in the centre of the larger one of which necrosis had occurred. In addition to the lymphocytic deposits in the nodules of the lungs there was considerable hemorrhage. The nodules in the mesentery showed no variations from the lesions already described. The ulcerations in the mouth were the result of a dense lymphocytic deposit with necrosis of the overlying mucous membrane. The nodule in the ileum was of a similar nature without ulceration. The lymphatic nodes showed follicular hypertrophy with more or less extensive lymphocytic deposits in the sinuses, in some cases entirely obliterating the structure of the node. In some of the larger nodes there were numerous small areas of necrosis. There was no endothelial or connective tissue proliferation and no giant cells were found.

Bacteriological examinations made by Dr. Bergey resulted in the following findings: From one of the nodules in the lungs the *Sarcina lutea*, *Micrococcus aureus*, and the *Bacillus diphtheria* were isolated. The diphtheria bacillus killed guinea-pigs in from forty-eight to seventy-two hours, with all of the typical lesions of experimental diphtheria. Cultures from one of the mesenteric lymph nodes showed a single colony of *Bacillus diphtheria*, conforming in all of its properties to the one isolated from the lungs. Cultures from the liver and spleen remained sterile.

REMARKS. In an analysis of this case a striking feature is at once appreciated in the lack of conformity between the blood picture it presented and the usual text-book interpretation of lymphatic leukemia. At no time during the disease did the total number of leukocytes exceed the normal, and with the exception of a slight increase in the percentage of the large mononuclear and transitional cells on February 20, the percentage of the various cells usually classed as lymphocytes at no time exceeded the normal. However, it has long been recognized by hematologists that an increase in the lymphocytes is not essential to a diagnosis of lymphatic leukemia. Türk,¹ in attempting to formulate a satisfactory classification of the lymphomatoses, divided lymphatic leukemia, both acute and chronic, into the following three varieties: (1) Lymphemic lymphatic leukemia, the common type,

¹ Türk, Wiener klinischen Wochenschrift, 1903, Nr. 39.

in which there is a high leukocytosis composed almost entirely of lymphocytes; (2) sublymphemic lymphatic leukemia, in which the total number of leukocytes is not materially increased, but in which the percentage of lymphocytes is increased; (3) alymphemic lymphatic leukemia, in which there is no alteration in the leukocytes as regards either their total number or the percentages of the individual varieties. This classification comprises all of the types of the disease ordinarily observed. When, however, we attempt to classify the case here reported according to Türk's scheme, it is seen that it does not conform to any one of the types. The total number of leukocytes was never above the normal, and the lymphocytes showed a continuous diminution in number until, on the day before death, they were only 1.6 per cent. of a total of 5200 leukocytes. In other words, there was a marked lymphopenia, and it is to express this condition that I have used the term "lymphopenic" lymphatic leukemia.

An appreciation of the variations which the blood picture in lymphatic leukemia may undergo can be best obtained by an understanding of a theory of Türk's, which, artificial though it be, is still very useful. He supposes that there is normally a definite ratio maintained between the production of lymphocytes and the rate of their extrusion into the blood channel. If, however, the tissues concerned in the production of lymphocytes become diseased, this ratio may become altered, so that the rate of extrusion of the lymphocytes becomes proportionately more rapid or less rapid than the rate of their production. Thus, for example, if there were some disease of the lymphatic tissues causing them to produce an abnormally large number of lymphocytes, and the rate of their extrusion were decreased, the lymphatic tissues would undergo great hypertrophy from the resulting retention of lymphocytes, but they would not be greatly increased in numbers in the circulating blood; if, on the other hand, the same increased production of lymphocytes occurred and the rate of their extrusion were increased, great numbers of them would appear in the circulating blood, but the hypertrophy of the lymphatic structures would be less marked than in the former case. Applying this theory to the case herein reported, we must assume that some influence incited the submucous lymphatic tissue of the mouth, the lymph nodes, the spleen, and possibly the bone marrow to an abnormally rapid production of lymphocytes, and at the same time the rate of their extru-

sion into the blood current was diminished so that there resulted a rapid hypertrophy of the lymphatic structures but an unusually small number of lymphocytes in the blood.

The marked preponderance of ulcerative mouth lesions during the earlier course of the disease is possible of two interpretations. They may be looked upon as simple ulcerative lesions that acted as a portal of entrance for the infectious agent producing the leukemia, or as merely an early manifestation of the leukemia itself. I, personally, incline to the latter view, first, because the base of this ulceration was seen to consist microscopically of masses of lymphocytes, and secondly, because it is a well-known clinical fact that the various portions of the gastro-intestinal tract are favorite seats for the deposit or proliferation of lymphatic tissue in the acute leukemias, the surface ulceration occurring as a result of this submucous proliferation. The history that the patient gave of the onset of symptoms shortly after having eaten heartily of pineapple, which he thought irritated his mouth, may have been simply the result of coincidence, though we are unable to exclude the possibility of this having instituted an ulcerative process that acted as a portal of entrance for the agent inducing the leukemia.

Concerning the etiology of the condition, no great amount of significance can be attached to the finding of spirochetes, fusiform bacteria, and streptococci in the ulcerated areas of the mouth, for these may be present in any severe inflammatory or ulcerative condition of the mouth. The finding of diphtheria bacilli in one of the nodules of the lungs and in one of the mesenteric lymph glands is somewhat more suggestive but by no means conclusive, for their presence may have been entirely of the nature of a secondary invasion. The improvement that the patient manifested on injections of diphtheria antitoxin can scarcely be attributed to any specific action, and little significance from the etiological standpoint can be attached to its occurrence.

Of decidedly unusual interest during the later course of the disease were the skin manifestations. These, shown by microscopic examination to be true lymphocytic deposits, gave the case a picture very like that of the "lymphoderma perniciosum" of Kaposi, recognized by its describer as being closely allied to leukemia and probably also to mycosis fungoides.

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A Study of the Eosinophilic Cell as Occurring in the Hematopoietic Organs in Diphtheria and Tuberculosis.

By GEORGE BURGESS FOSTER, M.D.

IN reviewing the literature on eosinophilia the earliest reference found is by Wharton Jones, in 1848. He states, in his essay entitled "The Blood Corpuscle Considered in its Different Phases of Development," that finely and coarsely granular leukocytes occur in the blood of man and most animals.

Subsequent writers—Rindfleisch, in 1863, Preyer, Förster, and others—described cells that were probably eosinophiles, but it may be stated with propriety that the existence of the eosinophile was not definitely shown until the publication of the article of Max Schultze in 1865. In 1868 Bizzozzero described elements in the red bone marrow which "resemble white blood cells and which contain accumulations of the fine fat granules in a portion of their (otherwise homogeneous) protoplasm." Neumann, Ponfick, Mosler, Henck, and Bieseadecki also published articles dealing with these cells in various conditions. In 1892 Neusser and his followers claimed that eosinophilia is due to stimulation of the sympathetic nervous system, but their findings were rather too radical to be thought of seriously by recent investigators.

Zappert's classical paper, "On the Occurrence of Eosinophilic Cells in Human Blood," was published in 1893, and has added much to our knowledge of the subject.

Among the more recent contributors to literature on the subject of eosinophilia, Opie, of Baltimore, whose three masterly articles have shed so much light on the problem, should be mentioned especially.

Simon, in "A Contribution to the Study of Eosinophilia," has enlightened investigators along the line, especially as to the eosinophilia associated with trichiniasis. The German literature of recent date has presented us with the work of Stchastnye. The vast majority of the investigations on the subject have dealt with the eosinophilic cell as it occurs in the circulating blood. In fact, in gleaning the writings of others for information as to the occurrence of these cells in the hematopoietic organs in fatal cases of diphtheria, the writer was able to find but one reference—the work of Mallory, Councilman, and Pearce, on the Pathology of Diphtheria. In this paper they assert that in their examinations of the thymus gland "brightly staining eosinophile cells were very numerous in all cases," and that in the lymph-nodes "a few eosinophiles are always found, and in some cases considerable numbers of them."

PERSONAL OBSERVATIONS. In June, 1906, while examining histologically the various tissues obtained postmortem from cases of contagious diseases in the Municipal Hospital, Philadelphia, the writer's interest was aroused by the enormous number of eosinophilic cells seen in the thymus gland of a patient dead of diphtheria. From this time on the thymus gland, spleen, and various lymph nodes were examined microscopically, to ascertain, if possible, the significance of the eosinophilia—whether it occurred in diseases other than diphtheria, from whence the cells came, at what time they appeared during the process, their role, and their ultimate end.

With this object in view, the investigation, which is recorded in the following pages, was begun. Autopsies were performed on forty-two cases. In the following table can be seen the variety of conditions met with:

Diphtheria, uncomplicated	14
Diphtheria and measles	3
Diphtheria and tuberculosis	2
Diphtheria and scarlatina	2
Diphtheria and ileocolitis	1
Diphtheria and enteric fever	1
Diphtheria, scarlatina, and measles	1
Scarlatina, uncomplicated	6
Scarlatina and rubella	1
Scarlatina and tuberculosis	1
Epidemic cerebrospinal meningitis	3
Tuberculous meningitis	1
Anthrax	2
Typhus fever	1
Dermatitis exfoliativa and tuberculosis	1
Cases of doubtful diagnosis	2

The tissues were in an excellent state of preservation. The subjects varied in age from seven weeks to fifty-seven years, as indicated below:

TABLE OF AGES.

Age.	Cases.	Per cent.
Under 1 year	4	9.5
" 1 to 2 years	5	11.9
" 2 to 3 "	4	9.5
" 3 to 4 "	6	14.2
" 4 to 5 "	3	7.1
" 5 to 6 "	1	2.4
" 6 to 7 "	2	4.8
" 7 to 8 "	3	7.1
" 8 to 9 "	2	4.8
" 9 to 10 "	4	9.5
" 10 to 15 "	1	2.4
" 15 to 29 "	2	4.8
" 29 to 39 "	1	2.4
" 39 to 69 "	4	9.5

METHODS. Small pieces of spleen, thymus gland, and palpable lymph nodes were taken and placed immediately in Zenker's fluid, in which they were left for twenty-four hours. They were then thoroughly washed in running water for twenty-four hours; after this they were taken through 70 per cent., 80 per cent., 95 per cent., and absolute alcohols, remaining in each twenty-four hours. Cedar oil and absolute alcohol, equal parts, followed by pure cedar oil, were used for clearing. The tissues were then infiltrated in three

paraffins and blocked. Sections were cut, as a rule, at 3 microns, although in a few cases, especially in tuberculous glands, it was necessary to cut them at 5 microns. They were then stained in 5 per cent. aqueous solution of eosin for one hour, washed in water, and placed in Unna's polychrome methylene blue (diluted 1 to 20) over night, usually about 18 hours. The sections were differentiated in glycerin-ether mixture (Grubler), diluted 1 to 15, until the blue ceased to come away. They were then washed in water, dehydrated in absolute alcohol, cleared in xylol, and mounted in xylol balsam. In sections where there was the least suspicion of tuberculosis, sections were stained by approved methods to demonstrate the tubercle bacillus. All sections were studied under the $\frac{1}{12}$ inch oil immersion lens, and the number of eosinophilic cells noted in a hundred consecutive fields recorded. The total number of cells was then divided by the number of fields examined, giving a factor, which, for convenience, will be called the eosinophilic index. This index is only approximately accurate because of the distribution of the eosinophiles in the tissues. In some of the specimens as many as twenty cells would be counted in one field, while in a field immediately adjacent not a single cell would be seen.

Sections of heart, lungs, liver, pancreas, kidney, and adrenals were studied in every case, but as the findings had no bearing on the subject under discussion, they will not be mentioned.

CHARACTERISTICS AND DISTRIBUTION OF EOSINOPHILES. Eosinophilic cells were always found, in varying numbers, in the lymph-adenoid structures, in all cases of diphtheria, and in each case where tuberculosis was present as an associated condition; but were absent in all the other diseases encountered. As a rule, the cells were more numerous in the thymus gland than in the other tissues. In individual cases, however, there did not appear to be any constant tissue of election. For example, in one case the spleen showed an eosinophilic index of 9, the thymus 0.71, and a postcervical lymph node 0.32; while in another case conditions were reversed, the thymus showing an index of 2.86, the spleen 0.40, and a peribronchial lymph node 1.38. Eosinophiles were never encountered in the heart, lungs, or kidneys; but they were observed in the pancreas once and in the adrenals in two other cases. In a pyelothrombosis of the liver, numbers of these

cells were demonstrable in the outer zone of the organizing thrombi and surrounding the thrombosed vessels.

The characteristics of the eosinophilic cells were essentially the same, irrespective of the location in which they were found. They varied considerably in size, some forms being little larger than the erythrocyte, and others showing all gradations of size from these up to those the size of a hyaline cell. Variations in shape were also observed. The majority of the cells were spherical in contour, but occasionally bizarre forms were seen. The nuclei were of two forms, one spherical and centrally placed, the other being polymorphous. In nearly all instances the latter was bi-lobed. The distribution of the granules in the cells varied greatly. In most cases they were evenly scattered throughout the protoplasm; but many of the cells showed a collection of the granules to one side. Free eosinophilic granules were commonly seen, and nearly all sections showed cells that had apparently ruptured, with discharge of their granules.

In the spleen it was not at all uncommon to find free eosinophilic granules, which had arranged themselves around certain mononuclear cells, and in many instances the granules were intracellular. The presence of eosinophilic granules in these cells would make them eosinophiles tinctorially; but it is doubtful that they were such functionally. It would seem that the presence of these granules in the mononuclear cells was due to a probable phagocytic property possessed by the cells, and, granting that they were phagocytic, it would be perfectly natural for them to englobulate any free granules with which they came in contact, as they would any other form of detritus. These same cells were also observed in the lymph nodes.

In the thymus gland many of the spindle-shaped connective-tissue cells contained scattered eosinophilic granules. Where collections of eosinophiles were found many of them were spindle-shaped, but in all other respects they resembled the regular type. In regard to a possible relationship between these cells and the connective-tissue cells it may be well to speak here of the theory of the origin of the eosinophile, as advanced by S. M. Stchastnye. In a recent article he states that the eosinophile is a product of hemolysis, and that these cells may be produced wherever hemolysis occurs. He bases this statement upon the belief that the fragments of the disintegrated

erythrocytes are gathered in by the phagocytic action of the mesenchyma cells and worked over so as to form the true eosinophile. Usually this takes place in the bone marrow, lymph nodes, spleen, and lungs because the largest accumulations of erythrocytes occur there. He also states in the same communication that "a host of authors claim for all microbes hemolytic properties," and he quotes Besredka, especially, as stating that streptococci associated with scarlatina have hemolytic properties. This latter statement, especially, militates against his views, for in most of the tissues studied personally from fatal cases of scarlet fever there was a marked accumulation of erythrocytes and numbers of streptococci, and yet not a single eosinophile was demonstrable. In two cases of anthrax hemolysis was evident; the organs were markedly congested and hordes of anthrax bacilli were present; yet no eosinophilic cells were found. Repeatedly in sections of spleen, especially, the conditions necessary, according to Stchastnye's hypothesis, have been encountered; but eosinophiles were absent unless the condition was that of diphtheria or tuberculosis.

Sections of all the lymphadenoid tissues, in which eosinophiles were present, showed these cells scattered diffusely through the organs, but there was a distinct tendency to grouping.

In the spleen eosinophiles were seen in greatest numbers beneath the capsule, in the lymph sinuses, and around the vessels. They were rarely seen within the splenic nodules, unless these bodies were the foci of degenerative or necrotic processes, in which event, numbers of eosinophiles were seen immediately surrounding the necrotic areas.

In the lymph nodes the distribution of the eosinophiles was much the same as in the spleen; but here the accumulation of these cells around the trabeculae was notable.

The points of predilection in the thymus seemed to be around the corpuscles of Hassel and in the vicinity of the connective tissue and vessels.

In not a few instances eosinophiles were seen within the vessels of the part and in the perivascular tissues. One section of spleen showed most beautifully eosinophiles in the vessel walls with one cell in the actual process of migration. It is difficult to say whether these cells were formed in the spleen and were passing into the circulation, or whether they were brought to the spleen in the circulation. Opie²

thinks that these cells are brought to the spleen from the bone marrow, as in inoculations upon animals he has found them two to four hours after inoculation, in the spleen, together with other elements characteristic of the bone marrow. Stchastnye⁴ believes that in a slowed circulation the granules arising from hemolysis pass through the vessel walls into the perivascular tissues, and are converted into eosinophiles, and that these eosinophiles may then pass back into the circulation. He stated, however, that he was never able to discern the granules in transition. Opie's view seems most reasonable, as in nearly every case some of these cells were undergoing karyokinesis.

Having observed these cells in their different aspects, the question naturally arose as to what was the factor that determined their presence in the hematopoietic organs? The antitoxin used in the treatment of diphtheria was thought of, but this was quickly dismissed, inasmuch as every case, regardless of its nature, admitted to the Municipal Hospital received an immunizing dose of diphtheria antitoxin to guard against the danger of house infection with that disease. Therefore, as an eosinophilia was not observed in cases of scarlatina and meningitis which received antitoxin, this serum was ruled out as a possible factor.

Then, in turning to the histories and autopsy notes of the different cases, several possible factors arose. Did the age of the individual play a part? Did the disease from which he suffered have any bearing? Did the clinical course of the disease in any way augment the phenomenon? What were the complications of the disease or the autopsy findings that might account for it? Could the day of the disease on which death occurred explain the condition?

Age was quickly thrown out as a cause, inasmuch as eosinophiles were found in cases ranging from seven weeks to thirty-four years.

It was found that the disease from which the patient suffered had an important bearing, as eosinophiles were observed in all cases of diphtheria and tuberculosis, but were absent in scarlatina, epidemic cerebrospinal meningitis, rubella, anthrax, and typhus fever, unless one of them was complicated by diphtheria or tuberculosis.

In considering the clinical course of the disease, the pulse and respiration obviously had no bearing, and were given no serious thought.

Several writers on hematology have shown that very high tempera-

tures tend to drive the eosinophiles from the circulation, and it was thought that this might explain their presence in the hematopoietic organs. The temperature ranged as high, however, in scarlatina and other diseases in which eosinophiles were absent, as in diphtheria and tuberculosis, where eosinophiles were present. Another point in reference to the temperature which was thought of was the preagonal rise or fall; but that, likewise, had no bearing.

Bronchopneumonia, myocarditis, and nephritis were the chief complications met with; but they occurred in the absence of eosinophilia as well as when it was present. The day of the disease on which death occurred threw little or no light on the subject.

Inasmuch as it is well known that an increase in the eosinophiles in the blood is augmented by helminthiasis, it was thought that the lumbricoid worms found at autopsy in some cases might contribute to the eosinophilia; but the *ascaris lumbricoides* was found in a case of meningitis which showed no eosinophiles.

Thus eliminating one factor after another, it was obvious that there was something in the virus of diphtheria and tuberculosis that controlled the phenomenon.

Simon⁵ states that tuberculin inoculations produce an eosinophilia in some of the lower animals, and Opie³ tells us that Noesske produced a local eosinophilia by inoculating animals with the tubercle bacillus, but failed with the pyogenic micrococci. Therefore the writer assumed that the toxins of the tubercle bacillus were responsible for the eosinophilia observed in those cases of the series reported here in which tuberculosis was an associated condition.

Not being able to find any references, in the literature at hand, as to the occurrence of an eosinophilia following injection of diphtheria toxins, it was decided to try inoculations upon guinea-pigs, to ascertain whether diphtheria toxins exercised an effect similar to those of the tubercle bacillus on the eosinophilic cells. Dr. Stewart, of the Bureau of Health laboratories, kindly furnished me with the toxin used.

INJECTION OF DIPHTHERIA TOXIN. Weight of guinea-pig, 527 grams. Inoculated in abdominal wall with 0.01 c.c. of virulent diphtheria toxin (Philadelphia Board of Health, No. 240). Pig began to look ill and refused to eat forty-eight hours after inoculation; grew progressively worse, and died in four days.

Autopsy Findings. Localized peritoneal exudate just below focus of inoculation; hypostatic congestion and edema of lungs; cloudy swelling of heart; congestion of spleen; fatty degeneration and punctate hemorrhages in adrenals; acute diffuse nephritis; cloudy swelling of liver.

Microscopic Examination. Abdominal wall at site of inoculation showed mostly granulation tissue around which were numerous eosinophiles. On the peritoneum below this focus was a localized exudate composed principally of fibrin, in which were enmeshed large numbers of leukocytes. Numbers of eosinophilic cells (eight to ten in a field of the $\frac{1}{12}$ inch oil immersion lens) and myriads of small granules, which were so numerous that the sections appeared as if the brightly staining granules had been dusted over them, were seen in this exudate. These granules were eosinophilic, and, as numbers of free nuclei were seen in the exudate, the inference is that they arose from disintegration of the eosinophiles.

It will be seen that the results of this experiment justified the inference that the presence of the eosinophiles might be due to a chemotactic influence exerted by the toxins of the Klebs-Loeffler organism. Dr. A. C. Abbott tells the writer that while working on experimental diphtheria, a number of years ago, he observed collections of eosinophilic cells upon the omentum of guinea-pigs inoculated, via the testicle, with diphtheria organisms. This still further tends to show that this organism elaborates a product or products which influence eosinophilia.

Satisfied that the toxins of tuberculosis and diphtheria exercise a specific action on the eosinophile, the next thought was as to why the collection of eosinophiles occurred in the hematopoietic organs? It is thought that these organs act as filters, as it were, through which bacterial toxins and other noxious agents are removed from the economy, and it is here that the battle between the offending material and the body cells is fought. Therefore, it seems perfectly natural that these organs should be the site of the eosinophilia a priori, as they evidently play a part in antagonizing the toxins.

This brings us to the consideration of the role of the eosinophile. Of this nothing is definitely known, and at best it is but a matter of conjecture. In this study many things have been observed that are at least suggestive, and perhaps they are worthy of note.

The arrangement of the eosinophiles around the focus at which the diphtheria toxin was injected in the experiment above recorded, their predilection for the hematopoietic organs, and their constant occurrence in the thymus gland, the internal secretion of which is thought to have an influence in combating infection (according to Osler²⁸), would suggest that their role, in diphtheria and tuberculosis, at least, is to antagonize the toxins of the disease.

Since Wright²⁰ began his classical studies in immunity much has been written on the subject of opsonins and antibodies in the body fluids. Metchnikoff,⁷ while admitting that there are sensitizing substances in these body fluids, still clings to his theory of immunity and claims these substances are elaborated by the leukocytes. "Some time ago," he says, "Pfeiffer and Marx proved that the sensitizing substance originates in three groups of organs—spleen, bone marrow, and lymphatic glands, or the phagocytic organs, for all these organs, without exception, contain and produce phagocytes.

"In corresponding researches Deutsch-Ditre proved the phagocytic organs are the nuclei of the antityphoid sensitizing substance.

"On the strength of these researches we may conclude that the phagocytes are able to elaborate and even to excrete into the blood substances which fix themselves on to the microbes and render them more amenable to destruction by the body.

"Acquired immunity is, therefore, superactivity of the phagocytes, which manifests itself by the overproduction of sensitizing substances, by their power of reacting strongly toward the introduction of microbes and their products, and lastly, by their capacity of enveloping pathogenic microbes and destroying them intracellularly."

Simon³ believes that the eosinophile is a glandular structure in which a secretory product is elaborated, inasmuch as detailed study, with adequate stains, such as eosinate of methylene blue, shows that the majority of the eosinophilic granules are in reality little vesicles, composed of a deeper staining outer wall and lighter staining contents, which he believes to be the specific secretory product. Correlating the belief of Metchnikoff, that the leukocytes do secrete antibodies; the claim of Simon, that the eosinophilic granule is in all probability capable of secretion; and the fact, as shown in this study, that the toxins of diphtheria and tuberculosis evidently exert a chemo-

tactic influence on the eosinophile cell—is there not a suggestion that the role of the eosinophilic cell is to secrete a substance antagonistic to certain toxins?

To the writer it seems probable that such is the case, although he realizes that his experience has been far too slight and that the studies recorded in this paper have been within too narrow limits to make such a statement justifiable. Nevertheless, it has long been thought that the eosinophilic cell plays an important part in combating infection. Hardy and Wesbrook³³ demonstrated that, when organisms were introduced into the intestines of animals, eosinophiles migrated into the canal and disappeared from the intestinal wall. Noesske³ produced an artificial eosinophilia with the tubercle bacillus. Howard and Perkins³⁹ demonstrated eosinophiles in appendicitis, salpingitis, and various other processes, inflammatory in character. Eosinophiles have been observed in neoplasms, and were found by the writer in the embryonic tissue of organizing thrombi in the hepatic vessels, and in numerous instances surrounding areas of necrosis in the spleen. Whether they were here to combat bacterial toxins or endogenous toxins elaborated by the disintegration of body cells cannot be stated. Opie² says: "Bacteria exert a chemotactic influence upon cells with eosinophilic granulation, attracting them from the blood to the site of inoculation and from the bone marrow to the blood. Though rarely phagocytic, they have a part in the changes following bacterial invasion." Still another fact that would go to show that the eosinophile is amenable to the chemotactic influence of certain bacterial toxins is their constant occurrence below mucous surfaces, where organisms are always present, as in the gut, and their infrequency in the deeper tissues unless these tissues are the foci of inflammatory or necrotic processes.

It would seem that the action of the eosinophile is a specific one, inasmuch as from personal observations the eosinophile seemed to be amenable to the chemotactic action of only the tubercle bacillus and the Klebs-Loeffler organism. It was shown a number of years ago by Widal and Ravaut, in their article on cytodiagnosis, that in certain conditions some one variety of cell predominated, as the lymphocyte in tuberculous effusions; and it would seem that we have an analogous condition here, the toxins in diphtheria and tuberculosis;

attracting the eosinophiles to the hematopoietic organs, while the toxins in the other conditions met with apparently exerted no influence, or at most a negative one. From the number of cases studied the observations should be conclusive in diphtheria and rather suggestive in tuberculosis. The number of cases of the other diseases studied is wholly inadequate to say conclusively that eosinophiles do or do not occur in the hematopoietic organs, but they may open up avenues for future investigation.

ANIMAL INOCULATIONS. With the object of finding out the evolutions of the eosinophile in combating infection, it was decided to inoculate several guinea-pigs with diphtheria organisms.

The culture used was isolated from a blood serum growth taken from the throat of a child ill with diphtheria. Tested on a pig, it was found to be very virulent. Transferred to neutral bouillon, there was a luxuriant growth in twenty-four hours, and this growth was used in the experiments.

The pigs chosen were of practically the same weight (350 to 360 grams), had been kept under the same hygienic surroundings, and were of identical strains. Each pig, except a control, was inoculated intraperitoneally with 0.5 c.c. of bouillon culture. The animals were killed fifteen minutes, one hour, two hours, four hours, six hours, and twenty-four hours, respectively, after inoculation. Killing instantly was deemed preferable to death by chloroform or other chemical agents, as it was thought that these substances might produce changes in the body fluids which would militate against the accuracy of the results. The peritoneal cavity was immediately opened, and smears were made from the same region in each case—the right iliac fossa. This region was chosen because the fluid was slight in amount and tended to gravitate here.

The spleen was placed in Zenker's fluid and prepared by the same technique as the tissues obtained at autopsy in the human cases, as stated in the first part of this paper. Smears were made from the bone marrow of the femur, and, with the smears from the peritoneum, were stained with Wright's blood stain.

The smears from the bone marrow showed such enormous numbers of eosinophiles that it was impossible to make even an approximate estimate of the comparative numbers in the different preparations.

The spleen of the control pig showed an eosinophilic index of 7.2; but Opie² states that eosinophiles are normally found in the spleen of the guinea-pig. The spleens in the fifteen-minute, one-hour, two-hour, four-hour, six-hour, and twenty-four-hour experiments showed eosinophilic indices of 11.4, 7, 9.8, 1.5, 4.75, and 5.25, respectively.

A differential count of the various leukocytes found in the peritoneal exudates of the respective pigs was then made. Atypical cells were found in considerable numbers, and great difficulty was experienced in deciding under which heading to classify them. Several spreads of normal guinea-pig blood and peritoneal fluid were studied, therefore, to enable the writer to become familiar with the cells found normally.

The mononuclear cells, which were larger than the erythrocyte, were classified as large lymphocytes, and those up to the size of an erythrocyte, as small lymphocytes. Two distinct types of eosinophiles were observed. Those of the first class were about the size of a large lymphocyte and identical with the eosinophiles found in the blood; were polymorphonuclear, and contained large regular granules, which stained brightly with eosin. The eosinophiles of the second class were smaller than those of the first class, their granules were more compact, stained less intensely with eosin, and showed one small, eccentrically placed, round or oval nucleus in most instances; although polymorphonuclear forms were not uncommon. These eosinophiles of the second class were also phagocytic.

In the fifteen-minute exudate the only phagocytes were large endothelial cells. In the one-hour exudate 25 per cent. of the eosinophiles of the second class, many endothelial cells, and polymorphonuclear neutrophils acted as phagocytes. The two-hour exudate showed 20 per cent. of the eosinophiles containing bacteria, and 15 per cent. of the neutrophils were phagocytic. A few large lymphocytes were also found to be phagocytic, while the endothelial cells were fewer in number and did not show as high a phagocytic index as in the previous specimens. No free bacteria or bacterial inclusions were seen in the four-, six-, or twenty-four-hour specimens. In the twenty-four-hour exudate many of the eosinophiles showed a partial polychromatophilia.

It is interesting to note that in the four-hour exudate, after phagocytosis had evidently ceased, the type of eosinophiles changed from those of the second class to those of the first class.

TABLE OF DIFFERENTIAL COUNTS OF LEUKOCYTES IN PERITONEAL EXUDATES.

Small lymphocytes	43.0	58.5	70.0	00	19	00
Large lymphocytes	43.5	40.0	18.5	6	24	00
Polymorphonuclears	1.5	0.0	4.5	21	6	77
Eosinophiles, first class . . .	1.0	0.0	0.0	72	51	13
Eosinophiles, second class. . .	11.0	1.5	7.0	1	00	10

It will thus be seen that the first effect of inoculation was to drive the eosinophiles from the peritoneal fluid, as evidenced in a study of the fifteen-minute exudate. It is also interesting to note that accompanying this temporary disappearance of the eosinophiles from the peritoneum a rise in the eosinophilic index occurred in the spleen. In the four-hour exudate, where the peritoneal eosinophilia reached its fastigium, the eosinophilic index of the spleen reached its lowest point. It would, therefore, seem that the presence or absence of eosinophiles in the peritoneal fluid varied inversely with the findings in the spleen.

Combining these results with those of the previous experiment, and with the facts elicited by study of the forty-two cases mentioned in the first part of this paper, the writer has formed the following conclusions:

CONCLUSIONS. 1. The occurrence of eosinophilic cells is constant in the hematopoietic organs in diphtheria and tuberculosis.

2. The toxins of the diphtheria and of the tubercle bacillus exert a positive chemotactic action on the eosinophile cell.

3. The chemotactic stimulus which attracts the eosinophile is a selective one, and is not possessed by all bacterial toxins, as eosinophiles were not found in the hematopoietic organs, in scarlatina, cerebrospinal meningitis, anthrax, rubella, or typhus fever.

4. The eosinophile elaborates either sensitizing substances or antibodies, which antagonize certain bacterial products, at least the toxins of diphtheria and tuberculosis.

5. Following intraperitoneal inoculations in guinea-pigs with the diphtheria bacillus, the number of eosinophiles in the spleen varies inversely with the number found in the peritoneal fluid.

6. In the guinea-pig the eosinophile cell is phagocytic for the Klebs-Loeffler bacillus.

The pleasant duty now remains of acknowledging my indebtedness to Dr. Randle C. Rosenberger, of the Jefferson Medical College

laboratories, who first suggested studying the hematopoietic organs in these infections.

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The Leukocytes in Diphtheria before and after the Administration of Antitoxin.

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THE 13 cases selected for this series were cases which, on admission to the Municipal Hospital, were regarded, clinically, as undoubted cases of diphtheria. These diagnoses were confirmed bacteriologically in all but one case (Case V).

Examinations of the blood were made as soon after admission as possible, in order to ascertain in each case the condition of the blood before the administration of antitoxin. In one case (Case XI) it was learned later that the patient had received a small dose of 3000 units about twenty-four hours before admission.

A second examination of the blood was made at varying periods after the first injection of antitoxin, in order to determine the effect on the leukocytes. The earliest examination after the injection, was made in two hours and thirty-five minutes; the latest in nineteen hours and fifty minutes.

In 10 of the 13 cases a third examination, at varying periods of time after the final dose of antitoxin had been administered, was made. The earliest of these counts was one hour after the last injection; the latest fifty hours and fifteen minutes.

The blood was taken from the lobule of the ear (except for the third count in Case XI) after cleansing with alcohol and drying. Puncture was sufficiently deep to produce free flow of blood, and the estimation of the leukocytes made with the Thoma-Zeiss apparatus, the diluent being 0.5 per cent. acetic acid. Smears were made, marked, and put away for future differential counting. Although the cases were studied in the spring of 1906, circumstances prevented differential counting until the fall of 1907. Five hundred cells were counted, except in the third count, in Case VIII, where 250 were counted. Wright's stain was used except in a few instances, where Ehrlich's tri-acid mixture was employed. Classification of the cells into the four great groups, polymorphonuclears, small and large lymphocytes, and eosinophiles, was employed as far as practicable. Cells with indented and kidney-shaped nuclei were classified as either polymorphonuclear neutrophiles or lymphocytes, according to the character of their protoplasm. The "Reizungsformen" of Türk,¹⁶ which he has described as occurring in infectious diseases, have been included as large lymphocytes, partially because these cells are not sharply differentiated from the lymphocytes, either in their morphology or tinctorial characters, and partly for the sake of simplicity.

Of the primary counts, 2 were made on the second day of disease, 4 on the third day, 3 on the fourth day, and 4 on the fifth day. The following table will show that, although the extreme high counts were obtained on the third and fourth days of disease, in a general way the counts are fairly uniform, all showing a moderate leukocytosis.

PRIMARY COUNTS IN REFERENCE TO THE DAY OF DISEASE.

		<i>Second Day.</i>	
Case I	18,000 leukocytes.
Case II	16,400 "
		<i>Third Day.</i>	
Case III	28,000 leukocytes.
Case IV	15,800 "
Case V	15,800 "
Case VII	14,400 "

<i>Fourth Day.</i>	
Case II	16,000 leukocytes.
Case VIII	29,800 "
Case IX	18,600 "
<i>Fifth Day.</i>	
Case VI	17,200 leukocytes.
Case XI	17,600 "
Case XII	16,800 "
Case XIII	14,700 "

All these cases showed exudate, varying, however, in amount; all were considerably prostrated by the disease, and apart from the amount of exudate would be considered severe cases, although only 3, with primary counts of 15,800, 17,600, and 29,800, the last complicated by nephritis, resulted fatally. Just how far the extent of the exudate influences the leukocytosis, varies in the minds of different authorities. In my series the case showing the most extensive exudate had, on his admission on the fifth day, a leukocytosis of only 17,600. Six cases in which the exudate was limited to the structures of the fauces, showed leukocytoses ranging between 15,800 and 18,600.

TABLE SHOWING RELATION OF LEUKOCYTOSES TO EXTENT OF EXUDATE.

<i>Faucial, Nasal, and Laryngeal Exudate.</i>	
Case XI	Fifth day of disease 15,800 leukocytes.
<i>Nasal and Faucial Exudate.</i>	
Case IV	Third day of disease 15,800 leukocytes.
Case VI	Fifth day of disease 17,200 "
Case VII	Third day of disease 14,400 "
Case VIII	Fourth day of disease (complicated by nephritis) 29,800 "
<i>Faucial and Laryngeal Exudate.</i>	
Case III	Third day of disease 28,000 leukocytes.
Case XIII	Fifth day of disease 14,700 "
<i>Faucial Exudate.</i>	
Case I	Second day of disease 18,000 leukocytes.
Case II	Fourth day of disease 16,000 "
Case V	Third day of disease 15,800 "
Case IX	Fourth day of disease 18,600 "
Case X	Second day of disease 16,400 "
Case XII	Fifth day of disease 16,800 "

During the course of the disease all the cases showed a diminution in the extent of the exudate except 2, and the whole counts of the leukocytes fell in 7 cases, but rose in 6 others. The first case which showed increase in the extent of exudate (Case XII) was one of faucial disease, which on the seventh day showed a nasal involvement, but which, nevertheless, on the eighth day showed a diminution of leukocytosis. Case XI was admitted with croup, but did not need intubation until about six hours after admission, in the course of which time the leukocytes advanced from 17,600 per c.mm. to 19,000 per c.mm. Of course, in this case the necessity for intubation does not necessarily presuppose increase in laryngeal exudate, but in order not to be considered prejudiced, that assumption should be allowed in this instance. Hence, in spite of the fact that Ewing⁷ states that the leukocytosis "frequently corresponds to the extent of local lesion," my series gives no grounds for believing this to be true. As Türk¹⁷ nicely puts it, "the blood findings in a case of infectious disease is not the expression of a single factor, the sort and severity of the infection, but it is in addition to this essentially dependent on the individuality of the attacked organism, its resisting and reacting powers."

The influence of temperature on the degree of leukocytosis is not in evidence, either in reference to the individual case or the series. The highest initial count, 29,800, showed a temperature of 100.2°. The highest initial temperature, 101.6°, showed a leukocytosis of 16,400. The variations in the other cases may be seen in the following table:

TEMPERATURE AND LEUKOCYTE COUNTS.

	Temperature.	Leukocytes.
Case I	100.4	18,000.
	100.0	20,400.
	100.0	16,800.
	100.4	20,000.
Case II	98.0	16,000.
	99.0	20,800.
	101.0	14,800.
Case III	101.0	28,000.
	103.0	19,400.
	99.8	17,000.
Case IV	98.6	15,800.
	97.0	9,600.

TEMPERATURE AND LEUKOCYTES COUNTS.

	Temperature.	Leukocytes.
Case V.	98.0	15,800.
	98.0	14,200.
Case VI	100.0	17,200.
	101.4	16,200.
	99.4	9,000.
Case VII.	99.2	14,400.
	100.4	17,400.
Case VIII	100.2	29,800.
	99.2	27,800.
	99.2	20,000.
Case IX	99.2	18,600.
	98.8	13,000.
	97.8	12,200.
Case X	101.6	16,400.
	102.4	19,800.
Case XI	100.0	17,600.
	101.0	19,000.
Case XII	100.0	16,800.
	99.6	18,800.
Case XIII	98.4	14,700.
	99.2	10,000.
	100.4	19,800.

The variations at different times in the same patient show that the leukocytosis and temperature have changed in a generally parallel manner in twelve instances, and have shown the opposite relation in nine instances. Hence I am led to conclude that, although fever in cases of toxic leukocytosis is believed to be approximately proportionate to the degree of leukocytosis, yet this relation cannot be accepted unqualifiedly as applying to cases of diphtheria.

In how far the administration of antitoxin vitiates these conclusions will be seen as I proceed. By reference to the counts attached to the cases at the end of this report, it will be seen that after the first dose of antitoxin the whole counts rose in 6 cases and fell in 7 others. Of the fatal cases, 2 showed a fall and 1 showed a rise. Nine cases were counted a third time, after the entire dosage of antitoxin had been

administered. Of these, 7 showed a fall below both initial and second counts; 1 showed a fall below the second, but not below the initial count; and 1 showed a rise above both primary and secondary. Hence it is seen that there is a decided tendency to the final drop of leukocytosis after the entire dosage has been administered. Whether or not this is due to antitoxin can only be determined by control cases, which, of course, could not be obtained in a community which so strongly favors antitoxin as a curative method as our own. Billings,¹ however, presents a series of cases which were not treated by the injection of antitoxin, and these cases show, during the course of the disease, the same general fall in leukocytes as that seen in my series treated with antitoxin.

The hypoleukocytosis following about thirty minutes after the injection of antitoxin, pointed out by Ewing,⁷ and later shown experimentally in rabbits by Morse,¹² cannot be seen in my series, possibly because none of my counts were made soon enough after the injection, the earliest being two hours and thirty-five minutes after the first injection. In that case the leukocytes had advanced from a primary count of 16,800 to 18,800.*

In no case in my series was a true hypoleukocytosis noted. Next to the lowest count made was the second in Case IV, 9600, taken one hour and thirty minutes after the third dose of antitoxin. This case, however, was extremely toxic and moribund at the time. Case IV showed 9000 leukocytes per c.mm. on the eighth day of disease, twenty-four hours and forty-five minutes after the last dose of antitoxin, and, although a severe case on admission, seemed to be in excellent general condition at the time of this count.

If it is true that hypoleukocytosis occurs very shortly after antitoxin injections, the return to a moderately high leukocytosis must be very rapid.

Broadly assuming that hypoleukocytosis means 8000 to 10,000 per c.mm., increases in two hours and thirty-five minutes to 18,000, in

* During the course of the general discussion following the reading of this paper, Mr. Weston, student assistant in the Laboratories of the Municipal Hospital, stated that in the course of counts made by himself every ten minutes for one hour after antitoxin administration, he had noted a fall of the leukocytosis for the first thirty or forty minutes, but that in the course of many counts he had never noted a true hypoleukocytosis.—H. T. K.

four hours to 19,000, in five hours to 19,800, and in seven hours and twenty minutes to 19,400, must have taken place very rapidly. Dehne and Hamburger⁴ have shown experimentally in man that the resorption of diphtheria antitoxin in such quantities as to be demonstrable in the blood serum is very slow, and that sometimes it is not complete until the third day after injection. Hence we should not look for marked changes in the corpuscular elements so quickly after the injection. *A priori*, hypoleukocytosis is not to be expected.

In this connection Ewing⁷ makes the further statement that in "favorable cases after the injection of antitoxin the leukocytosis never again reaches its original height." That this statement is not true is seen by reference to Cases I, II, III, VII, X, XII, all of which resulted favorably and all of which showed an advance of leukocytosis at the second count.

Bize² finds that the failure of the leukocytes to show definite changes after antitoxin administration, is due to insufficient dosage. This can hardly be the cause in my series, since the total doses ranged as follows: In 1 case, 5000 units; in 3 cases, 7500 units each; in 1 case, 15,000 units; in 1 case, 17,500 units; in 2 cases, 22,500 units each; in 4 cases, 30,000 units each; and in 1 case, 43,000 units.

POLYMORPHONUCLEAR NEUTROPHILES.

The percentage of polymorphonuclear neutrophiles in this series ranges between 75 and 79 per cent. in the vast majority of instances, an isolated instance running down to 69.2 per cent., another up to 87.6 per cent. This general tendency has seemed to me, from a theoretical standpoint, to show a rather low polymorphonuclear leukocytosis in relation to the moderately high general leukocytosis. On reading the counts in some other diseases, however, and particularly on the basis of a personal statement of Dr. J. C. Da Costa, Jr., I am finally led to believe that this is by no means peculiar to diphtheria, but commonly occurs in other toxic leukocytoses. In those cases in which the initial count shows a relatively high polymorphonuclear leukocytosis the ratio is maintained in a general way in the subsequent counts, as is strikingly exemplified in Case X, in which the polymor-

phonuclear percentages were successively 87.6 per cent., 84 per cent., 85 per cent. Although the rule is that these percentages in the greater number of the cases vary within only narrow limits, yet in 5 cases they varied in the degree of 5 per cent., of which 4 cases showed increases: 3 without any explanation for the relatively high increase, the fourth showing the development of bronchopneumonia at the time of the later counts 1; one showed a decrease, but was, at the time of the count, however, moribund.

Reviewing the cases as a whole, we find that 6 showed an increase and 7 a decrease in the polymorphonuclear percentages in those counts which were made after the first dose of antitoxin. In these same counts computations of the actual number of polymorphonuclear neutrophils per cubic millimeter show an increase in 7 cases and a decrease in 6 cases.

In those cases in which a third count was taken, 6 showed an increase and 4 a decrease in percentage; 3 showed an increase, 6 a decrease, in actual numbers.

This review will show that, as Cabot³ says, "the proportion of polymorphonuclear cells is usually directly proportional to the total increase of leukocytes." In addition my cases show that the administration of antitoxin exerts no determinable influence either on the whole number or on the percentage of the polymorphonuclear cells.

MONONUCLEAR CELLS.

Inasmuch as the numbers of eosinophiles, myelocytes, and mast cells are small, the proportion of mononuclear cells and their variations after antitoxin administration can be justly estimated by simple reference to the percentage of polymorphonuclears. Hence I shall not enter into detailed discussion of these cells.

My counts in some instances show a relatively high percentage of large lymphocytes as compared with the smaller forms, but I do not feel warranted in drawing conclusions from this, because the line of demarcation between the small and large lymphocytes (including the so-called large mononuclear leukocytes) is so much a matter of personal equation that, although my counts agree with those of many

others, they are at wide variance with some few who have found a great preponderance of smaller forms. In my own classification I have considered staining properties of nucleus and protoplasm as well as morphology and size.

Ewing's⁷ counts show 2 cases in which the lymphocyte percentages were 60 and 62 in leukocytoses of 72,000 and 22,500, and Rieder¹⁵ reports a case in which the proportion of mononuclear cells, most of which he says were small lymphocytes, reached 63.9 per cent. This count was made on the eighth day of disease; the case had been free from fever for several days, and had only 9,400 leukocytes per c.mm. at the time. The same case, with a leukocytosis of 23,000 the first day of disease, had shown only 19.6 per cent. of mononuclear cells. From these counts I have computed the absolute numbers, and find that the mononuclears in this time decreased from 4586 to 4006.

These exceptional cases, less than 1 per cent. of the cases that have been reported up to date, can hardly interfere with my general conclusion, that, inasmuch as the mononuclears range in direct ratio with the polymorphonuclears, there is nothing peculiar in the number or percentage of mononuclear cells in diphtheria, and they show no demonstrable change after the administration of antitoxin.

EOSINOPHILES.

The work of Foster,⁸ in which he has demonstrated the striking abundance of eosinophiles in the hematopoietic organs in cases of diphtheria, lends added interest to the study of their presence in the blood of such cases. Whatever may be the cause of this accumulation in these solid tissues, there can be no doubt that, even in spite of isolated reports of high percentages, the percentage of eosinophiles in cases of diphtheria is remarkably low. The cases of Meyer,¹¹ Rieder,¹⁵ Reckzeh,¹⁴ Morse,¹² Billings,¹ Engel,⁶ and others show this low percentage in contrast to the generally accepted statement of Zappert,¹⁸ that children in health usually show higher counts of eosinophiles than are found in adults. Of my cases the primary counts show eosinophiles in only 7 of the 13 cases. Of these, 5 were less than 1 per cent. (0.2 per cent., 0.2 per cent., 0.6 per cent., 0.6 per cent.

0.8 per cent.), one was 1.4 per cent., and one 3.2 per cent. In response to the dictum of Ehrlich,⁵ we look for a diminution in the eosinophiles in most of the acute leukocytoses, but hardly so marked and persistent a diminution as we see in diphtheria, even after the leukocytosis diminishes. High fevers (Zappert¹⁸) produce low percentages of eosinophiles, but high grades of fever certainly exerted no influence in my counts, as many were made below 100° F. It would appear as the most plausible explanation that diphtheria brings about certain changes which either render the blood negatively chemotactic or the hematopoietic organs positively chemotactic for eosinophiles, with the result that they practically disappear from the circulating blood during the early course of the disease.

After the first dose of antitoxin the percentage and absolute number of eosinophiles were diminished in 6 cases and increased in 3, showing no change in 4. Three cases showed no eosinophiles at any time. Of the 6 cases that showed diminished eosinophiles, 3 had increased temperatures, 3 had decreased temperatures at the time of counts, 2 showed increase in the extent of exudate, and 4 showed decrease.

Of the 8 cases which showed eosinophiles on the third count, 4 were higher, 4 were lower, in percentages of eosinophiles, than those of the initial counts. The same was true in reference to absolute numbers.

Pitkianin¹³ sums up the status of eosinophiles in the blood of diphtheria patients as follows: "In the early days of the disease the eosinophiles are completely or almost completely absent from the blood, only to reappear in the later days." Careful examination of the eosinophiles in my own cases fails to show that antitoxin in any way affects these cells.

MYELOCYTES.

Repeated in the text-books so frequently that they have become almost classic in the hematology of diphtheria, the statements of Engel⁶ in reference to the presence of myelocytes in the blood of diphtheria, and his conclusions in reference to their number in the individual case, demand attention. These cells were present in most of his 25 favorable cases in quantities as high as 1.5 per cent. In his 7 fatal cases they ranged between 3.6 and 16.4 per cent. He fixes 2

per cent. as the border-line, and gives a bad prognosis to cases over that percentage, not, however, giving an unqualifiedly unfavorable prognosis to those which fall below 2 per cent. He speaks of this condition as "Myelocythämia infolge der Diphtherie." It is interesting to note the statement of Grawitz¹⁰ in a foot-note in which he says, in reference to these conclusions of Engel: "I cannot accede to this conception, since, as has been mentioned frequently, myelocytes are often met with in the blood in the leukocytoses of childhood." He then refers to the work of Reckzeh,¹⁴ in which 6 cases of diphtheria are reported, 3 showing 2 per cent. myelocytes and terminating favorably. In the work of other writers, reports of the presence of myelocytes are infrequent, and then only in small percentages. In the 37 counts which I made I found myelocytes in 8 counts, limited to 4 cases, all of which terminated favorably. Eosinophilic myelocytes were present in 2 counts, 0.2 per cent. in each. In only 2 counts did the neutrophilic myelocytes reach 0.6 per cent., all the others showing only 0.2 per cent. in each instance. In absolute numbers the variance was between 29 and 120 myelocytes per c.mm. Only 2 of the cases showed myelocytes in the initial counts, so it is impossible for me to draw definite conclusions as to the effect of antitoxin on these cells. Observation without statistical study would lead to the belief that such effect does not exist.

The reports of all writers, except Engel himself, furnish evidence to discredit his statements, and when I consider my own findings in connection with those of others, I conclude that myelemia is by no means constant in diphtheria; that the findings of Reckzeh preclude the conclusion that 2 per cent. or more of myelocytes give an unfavorable prognosis, and that the small numbers of myelocytes present in the vast majority of cases of diphtheria make it impossible with our present methods of study to draw conclusions as to the effect of antitoxin on these cells.

BASOPHILES.

Basophiles were present in 13 of the 37 counts, limited to 7 cases, and ranging between 0.2 per cent. and 0.6 per cent.; in actual numbers, between 33 and 112 per c.mm. In my own cases it will be seen

that basophiles are more frequent than myelocytes, but still in such numbers that I do not feel justified in drawing conclusions as to the effect of antitoxin on them.

STAINING REACTION.

The statements of Ewing, in reference to the staining reaction of the leukocytes in diphtheria, were based largely on his experience with gentian violet as a stain for purposes of differential counting in the Thoma-Zeiss chamber. This is no place to comment on this method of differential counting, and, as all my differentials were made in dry preparations, I am not prepared to either combat or confirm his statements. In my search of the literature I failed to find reference to any work which had been done on the same line. In dry preparations, however, Ewing found that there was a certain affinity for eosin on the part of some of the polymorphonuclears with the production of so-called pseudo-eosinophile cells.

Billings¹ believes that they are the result of "the staining methods, rather than that they exist as pathological entities." I stained my smears with Wright's stain, Ehrlich's tri-acid mixture, and hematoxylin and eosin, the last as a control, but in none of these was I able to discover any reaction on the part of the blood cells of diphtheria in any wise different from that of the cells in other similar diseased conditions.

CONCLUSIONS.

1. Diphtheria is accompanied by a varying degree of hyperleukocytosis, usually moderate. Occasionally hyperleukocytosis may be absent in extremely toxic or extremely mild cases.

2. The differential counts in the leukocytoses of diphtheria show proportions of polymorphonuclear and mononuclear cells quite consistent with the grade of leukocytosis. In these leukocytoses the eosinophiles are present in unusually small numbers, and the myelocytes and basophiles in moderately small numbers.

3. Neither the degree of leukocytosis nor the proportions of any of

its constituent types of cells indicates, except within very broad general lines, the severity of the infection nor the outcome of the disease.

4. The administration of antitoxin has no appreciable effect on the degree of the leukocytosis, the proportion of its constituent types of cells, or the staining reactions of these cells in dry preparations, stained by either Wright's method, Ehrlich's tri-acid mixture, or hematoxylin and eosin.

I wish to thank Dr. B. Franklin Royer, Dr. Allen J. Smith, Dr. C. Y. White, and Dr. J. C. Da Costa, Jr., for aid in the preparation of this report.

The blood counts in the various cases follow:

CASE I.—C. E., aged nine years. Admitted suffering from tonsillar diphtheria.

Nose: No exudate; no discharge; no crusts.

Throat: Both tonsils are covered with a thick, white exudate, which spreads on the left anterior pillar. The fauces are intensely congested; no punctation. The diphtheria ran an uneventful course, but on May 25 the patient developed a large antitoxin abscess in the left flank.

Culture: Positive.

Result: Cured.

(a) May 14, 1906, 4.30 P.M., second day of disease, 18,000 leukocytes, counted partly by Wright's and partly by Ehrlich's stain.

Polymorphonuclears	75.0 per cent.	13,500.0 per c.mm.
Small lymphocytes	15.2 "	2,736.0 "
Large lymphocytes	8.0 "	1,440.0 "
Eosinophiles	1.4 "	252.0 "
Basophiles	0.2 "	36.0 "
Myelocytes	0.2 "	36.0 "

(b) May 15, 1906, 11.15 A.M., third day of disease, 20,400 leukocytes. Fifteen hours and fifteen minutes after first dose of 7500 units:

Polymorphonuclears	79.0 per cent.	16,116.0 per c.mm.
Small lymphocytes	13.8 "	2,815.2 "
Large lymphocytes	6.8 "	1,387.2 "
Eosinophiles	0.2 "	40.8 "
Myelocytes	0.2 "	40.8 "

(c) May 16, 1906, 12.30 P.M., fourth day of disease, 16,800 leukocytes. One hour after last dose, in all, 17,500 units:

Polymorphonuclears	80.0 per cent.	13,440.0 per c.mm.
Small lymphocytes	9.8 "	1,478.4 "
Large lymphocytes	10.2 "	1,713.6 "
Eosinophiles	0.8 "	134.4 "
Basophiles	0.2 "	33.6 "

(d) May 17, 1906, 2.30 P.M., 20,000 leukocytes, counted partly by Wright's and partly by Ehrlich's stain. Twenty-seven hours after last dose, in all, 17,500 units:

Polymorphonuclears	81.6 per cent.	16,320.0 per c.mm.
Small lymphocytes	8.8 "	1,760.0 "
Large lymphocytes	8.6 "	1,720.0 "
Eosinophiles	0.4 "	80.0 "
Myelocytes	0.6 "	120.0 "

CASE II.—F. W., aged eight years. Patient admitted suffering from faucial diphtheria.

Nose: No exudate; no crusts; no discharge.

Throat: A thick, pultaceous, creamy-white exudate covers both tonsils, uvula, and anterior pillars, excluding the view of the pharynx. Uvula is elongated and swollen. There is moderate congestion; no punctation. Ran an uneventful course. Developed an erythematous antitoxin rash on May 19.

Culture: Positive.

Result: Cured.

(a) May 15, 1906, 1.45 P.M., fourth day of disease, 16,000 leukocytes, counted partly by Wright's and partly by Ehrlich's stain:

Polymorphonuclears	77.4 per cent.	12,384.0 per c.mm.
Small lymphocytes	11.8 "	1,888.0 "
Large lymphocytes	10.4 "	1,644.0 "
Eosinophiles	0.2 "	32.0 "
Myelocytes	0.2 "	32.0 "

(b) May 16, 1906, 9.45 A.M., fifth day of disease, 20,800 leukocytes. Nineteen hours and fifty minutes after first dose of 7500 units.

Polymorphonuclears	77.6 per cent.	16,140.8 per c.mm.
Small lymphocytes	13.2 "	2,745.6 "
Large lymphocytes	9.0 "	1,872.0 "
Basophiles	0.2 "	41.6 "

(c) May 18, 1906, 1.45 P.M., seventh day of disease, 14,800 leukocytes. Twenty-eight hours and fifteen minutes after last dose, in all, 22,500 units:

Polymorphonuclears	85.0 per cent.	12,580.0 per c.mm.
Small lymphocytes	7.0 "	1,036.0 "
Large lymphocytes	6.2 "	917.6 "
Eosinophiles	1.2 "	177.6 "
Basophiles	0.4 "	59.2 "
Myelocytes	0.2 "	29.6 "

CASE III.—S. J., aged four years. Admitted with faucial and laryngeal diphtheria and intubated before admission.

Nose: No exudate; no discharge; no crusts.

Throat: A thick, yellowish-white, smooth, soft exudate is spread over both tonsils, encroaching upon the anterior pillars and partly covering the uvula; moderate congestion; no punctation. Was extubated after six days, and recovered uneventfully.

Culture: Positive.

Result: Cured.

(a) May 17, 1906, 1.30 P.M., third day of disease, 28,000 leukocytes:

Polymorphonuclears	78.8 per cent.	22,064.0 per c.mm.
Small lymphocytes	9.8 "	2,744.0 "
Large lymphocytes	11.4 "	3,192.0 "

(b) May 17, 1906, 9.00 P.M., 19,400 leukocytes. Seven hours and twenty minutes after first dose of 10,000 units:

Polymorphonuclears	76.8 per cent.	14,899.2 per c.mm.
Small lymphocytes	9.8 "	1,901.2 "
Large lymphocytes	13.4 "	2,599.6 "

(c) May 20, 1906, 1.30 P.M., sixth day of disease, 17,000 leukocytes. Twenty-seven hours after last dose, in all, 30,000 units:

Polymorphonuclears	82.2 per cent.	13,974.0 per c.mm.
Large lymphocytes	9.6 "	1,602.0 "
Small lymphocytes	7.6 "	1,292.0 "
Eosinophiles	0.6 "	102.0 "

CASE IV.—H. D., aged five years. Admitted with faucial and nasal diphtheria.

Nose: Profuse mucopurulent, "dippy" smelling discharge; no visible exudate; no crusts; very little excoriation.

Throat: A thick, grayish-white, soft exudate fills the fauces; it covers the tonsils, uvula, pillars, so as to completely obscure them, and apparently completely shuts off the faucial opening, with slight surrounding congestion; no punctation. The child has a "bull" neck, markedly prostrated, and has the left border of the heart 2 cm. without the midclavicular line, with equality of intensity of first and second sounds.

Culture: Positive.

Result: Died, May 21, 1906.

(a) May 20, 1906, 1.00 P.M., third day of disease, 15,800 leukocytes:

Polymorphonuclears	77.0 per cent.	12,166.0 per c.mm.
Small lymphocytes	12.0 "	1,896.0 "
Large lymphocytes	11.0 "	1,738.0 "

(b) May 21, 1906, 2.30 P.M., fourth day of disease, 9600 leukocytes. Twenty-five hours and thirty minutes after first dose of 10,000 units, and one hour and thirty minutes after last dose, in all, 30,000 units:

Polymorphonuclear	75.0 per cent.	7,200.0 per c.mm.
Small lymphocytes	14.6 "	1,401.6 "
Large lymphocytes	10.2 "	979.2 "
Eosinophiles	0.2 "	19.2 "

CASE V.—L. G., aged twelve years. Admitted with tonsillar diphtheria.

Nose: No exudates; no discharge; no crust.

Throat: Both tonsils are much enlarged and are dotted with pea-sized spots of creamy-white, pultaceous exudate; moderate congestion; no punctation.

Cultures: On 21st, 22d, and 25th, negative.

Result: Recovery uneventful.

(a) May 21, 1906, 2.10 P.M., third day of disease, 15,800 leukocytes:

Polymorphonuclears	84.0 per cent.	13,272.0 per c.mm.
Small lymphocytes	6.6 "	1,042.8 "
Large lymphocytes	9.2 "	1,453.6 "
Myelocytes	0.2 "	31.6 "

(b) May 21, 1906, 10.15 P.M., 14,200 leukocytes. Seven hours and thirty-five minutes after only dose, 5000 units:

Polymorphonuclears	79.0 per cent.	11,218.0 per c.mm.
Small lymphocytes	10.2 "	1,448.4 "
Large lymphocytes	10.6 "	1,505.2 "
Mononuclear eosinophiles	0.2 "	28.4 "

CASE VI.—J. C., aged five years. Admitted with faucial and nasal diphtheria:

Nose: Exudate present in both anterior nares; profuse mucopurulent discharge; moderate excoriation.

Throat: Smooth surfaced, yellowish-white exudate covers both tonsils and extends forward on the anterior pillars. The soft palate is somewhat congested and punctate. On June 4, morbilliform antitoxin rash. On June 6 developed palatal paralysis and cardiac dilatation. On the 19th the antitoxin rash was hemorrhagic.

Culture: Positive.

Result: Discharged, cured, on July 18. Had albuminuria on June 7; no casts.

(a) May 22, 1906, 7.00 P.M., fifth day of disease, 17,200 leukocytes:

Polymorphonuclears	76.2 per cent.	13,106.4 per c.mm.
Small lymphocytes	13.2 "	2,270.4 "
Large lymphocytes	10.2 "	1,754.4 "
Eosinophiles	0.2 "	34.4 "
Basophiles	0.2 "	34.4 "

(b) May 23, 1906, 9.45 A.M., sixth day of disease, 16,200 leukocytes. Fourteen hours and forty-five minutes after first dose of 7500 units:

Polymorphonuclears	83.0 per cent.	13,446 per c.mm.
Small lymphocytes	12.0 "	1,944.0 "
Large lymphocytes	4.4 "	712.0 "
Eosinophiles	0.6 "	97.0 "

(c) May 25, 1906, 12.15 P.M., eighth day of disease, 9000 leukocytes. Twenty-four hours and forty-five minutes after last dose, in all, 25,000 units:

Polymorphonuclears	80.6 per cent.	7254.0 per c.mm.
Small lymphocytes	10.6 "	954.0 "
Large lymphocytes	6.8 "	612.0 "
Polymorphonuclear eosinophiles	0.8 "	72.0 "
Mononuclear eosinophiles	0.2 "	18.0 "
Basophiles	0.4 "	36.0 "
Myelocytes	0.6 "	54.0 "

CASE VII.—A. C., aged three years. Admitted with faucial and nasal diphtheria.

Nose: Exudate present in right nostril; profuse mucopurulent discharge; moderate excoriation.

Throat: Both tonsils are covered with thick, rough-surfaced, grayish-white exudate, which extends lightly on the anterior pillars. Moderate congestion; no punctation. On the 25th developed rubella. On June 11 developed albuminuria, but no casts. On June 15 showed marked diminution in the amount of urine. Persistence of the urticarial rash, which first showed itself on June 6.

Culture: Positive.

Result: Discharged on September 1, cured.

(a) May 22, 1906, 7.20 P.M., third day of disease, 14,400 leukocytes (counted with Ehrlich's tri-acid mixture):

Polymorphonuclears	78.4 per cent.	11,289.6 per c.mm.
Small lymphocytes	11.2 "	1,612.8 "
Large lymphocytes	9.8 "	1,411.2 "
Eosinophiles	0.6 "	86.4 "

(b) May 23, 1906, 10.15 P.M., fourth day of disease, 17,400 leukocytes (counted with Ehrlich's tri-acid stain). Fifteen hours and ten minutes after first dose, 7500 units:

Polymorphonuclears	78.0 per cent.	13,572.0 per c.mm.
Small lymphocytes	16.0 "	2,784.0 "
Large lymphocytes	5.8 "	1,009.2 "
Eosinophiles	0.2 "	34.8 "

Third count not made because child developed rubella.

CASE VIII.—H. C., aged five years. Admitted with faucial and nasal diphtheria.

Nose: Profuse mucopurulent discharge with flocculi. Exudate visible well back in right nostril.

Throat: A thick, soft, smooth, dirty white exudate covers both tonsils and base of uvula, and anterior pillars and part of soft palate. There is moderate congestion.

On admission was markedly prostrated, and four days after admission developed cardiac dilatation, vomiting, exhaustion, and on sixth day after admission died.

On May 29, the second day after he was admitted, the urine showed albumin, epithelial, leukocytic, and granular cases.

Culture: Positive.

Result: Died June 2, 1906.

(a) May 28, 1906, 11.00 P.M., fourth day of disease, 29,800 leukocytes:

Polymorphonuclears	79.0 per cent.	23,542.0 per c.mm.
Small lymphocytes	10.8 "	3,218.4 "
Large lymphocytes	10.2 "	3,039.6 "

(b) May 29, 1906, 11.00 A.M., fifth day of disease, 27,800 leukocytes. Eleven hours and five minutes after first dose of 10,000 units:

Polymorphonuclears	79.2 per cent.	22,017.6 per c.mm.
Small lymphocytes	7.8 "	2,168.4 "
Large lymphocytes	12.8 "	3,558.4 "
Basophiles	0.2 "	55.6 "

(c) May 31, 1906, 2.50 P.M., seventh day of disease, 20,000 leukocytes (differential count of 250 cells in dry preparations). Twenty-eight hours and fifty minutes after last dose, in all, 30,000 units:

Polymorphonuclears	74.0 per cent.	14,800.0 per c.mm.
Small lymphocytes	13.2 "	2,640.0 "
Large lymphocytes	12.8 "	2,560.0 "

CASE IX.—W. H., aged six years. Admitted with faucial diphtheria.

Nose: No exudate; no discharge; no crusts.

Throat: Both tonsils are covered with thick, soft, smooth, yellowish-white exudate, which extends forward on the anterior pillars and the sides of the uvula and on the right side to the soft palate. Moderate congestion; no punctation. On June 5, showed albuminuria and a few hyaline casts.

Culture: Positive.

Result: Discharged on June 21, cured.

(a) June 3, 1906, 1.00 P.M., fourth day of disease, 18,600 leukocytes:

Polymorphonuclears	77.2 per cent.	14,359.2 per c.mm.
Small lymphocytes	13.4 "	2,492.4 "
Large lymphocytes	8.6 "	1,599.6 "
Eosinophiles	0.8 "	148.8 "

(b) June 3, 1906, 6.20 P.M., 13,000 leukocytes. Four hours and fifty-five minutes after first dose of 7500 units:

Polymorphonuclears	79.6 per cent.	10,348.0 per c.mm.
Small lymphocytes	11.8 "	1,534.0 "
Large lymphocytes	7.6 "	988.0 "
Eosinophiles	0.4 "	52.0 "
Basophiles	0.6 "	78.0 "

(c) June 6, 1906, 12.15 P.M., seventh day of disease, 12,200 leukocytes. Two days, two hours, and fifteen minutes after last dose, in all, 15,000 units:

Polymorphonuclears	77.0 per cent.	9,394.0 per c.mm.
Small lymphocytes	10.4 "	1,268.8 "
Large lymphocytes	11.4 "	1,390.8 "
Eosinophiles	0.8 "	97.6 "
Basophiles	0.4 "	48.8 "

CASE X.—L. T., aged eight years. Admitted with faucial and tonsillar diphtheria.

Nose: No exudate; no discharge; no crusts.

Throat: Thick, dirty white, rough, soft exudate covers both tonsils and encroaches on the anterior pillars. Moderate congestion; no punctuation.

(On the third day after admission shows albuminuria, but no casts.

Result: Discharged, cured, one week after admission.

(a) June 4, 1906, 1.15 P.M., second day of disease, 16,400 leukocytes:

Polymorphonuclears	87.6 per cent.	14,366.4 per c.mm.
Small lymphocytes	7.6 "	1,246.4 "
Large lymphocytes	4.8 "	787.2 "

(b) June 4, 1906, 7.00 P.M., 19,800 leukocytes. Five hours after first dose of 7500 units:

Polymorphonuclears	84.0 per cent.	16,632.0 per c.mm.
Small lymphocytes	7.4 "	1,465.2 "
Large lymphocytes	8.6 "	1,702.8 "

(c) June 6, 1906, 11.55 A.M., fourth day of disease, 17,400 leukocytes. Twenty-four hours after last dose, in all, 15,000 units:

Polymorphonuclears	85.0 per cent.	14,790.0 per c.mm.
Small lymphocytes	7.2 "	1,252.8 "
Large lymphocytes	7.8 "	1,357.2 "

CASE XI.—S. K., aged five and one-half years. Admitted with nasal, faucial, and laryngeal diphtheria, and had been given about 3000 units of antitoxin about twenty-four hours before admission.

Nose: Both nostrils occluded with yellowish-white exudate; profuse, syrupy, floccular discharge. Herpes about nares.

Throat: Soft, thick, rough-surfaced, yellowish-white exudate covers both tonsils, anterior pillars, uvula, and extends far over on the right side of the soft palate. Exudate leaves a bleeding surface when removed. Child has a "bull" neck. Was intubated with great relief about six hours after admission. Two days after admission, became prostrated, had hemorrhage from the right nostril, which was controlled, but the child died several hours later.

The urine analysis showed albumin, epithelial, granular, and leukocytic casts.

Culture: Positive.

Result: Died 1.30 P.M., June 7, 1906.

Autopsy made by Dr. George Foster.

Anatomical Diagnosis: Bronchopneumonia, congestion, and edema of lungs; cloudy swelling of the heart and liver; acute parenchymatous nephritis.

Bacteriological Findings: Heart, *Streptococcus pyogenes*; liver, negative; right lung, *Klebs-Loeffler* and *Streptococcus pyogenes*; left lung, *Klebs-Loeffler*; kidney, negative.

Histological Diagnosis: Bronchopneumonia, congestion, coagulation necrosis of lungs; acute tubular nephritis; acute splenitis; congestion of liver.

(a) June 4, 1906, 1.35 P.M., fifth day of disease, 17,600 leukocytes. About twenty-four hours after dose of 3000 units, before admission:

Polymorphonuclears	77.0 per cent.	13,552.0 per c.mm.
Small lymphocytes	18.8 "	3,308.8 "
Large lymphocytes	4.0 "	704.0 "
Basophiles	0.2 "	35.2 "

(b) June 4, 1906, 6.45 P.M., 19,000 leukocytes. Four hours and forty-five minutes after first dose of 10,000 units in house:

Polymorphonuclears	78.0 per cent.	14,820.0 per c.mm.
Small lymphocytes	8.6 "	1,634.0 "
Large lymphocytes	12.8 "	2,432.0 "
Eosinophiles	0.2 "	38.0 "
Basophiles	0.4 "	76.0 "

(c) June 7, 1906, eighth day of disease. About twenty-four hours after last dose, in all, 43,000 units (child in dying condition). No evidence of circulation, and the child was too cyanosed for a dependable whole count. Smears were made by cutting a vein in wrist):

Polymorphonuclears	72.0 per cent.
Small lymphocytes	15.2 "
Large lymphocytes	12.6 "
Eosinophiles	0.2 "

CASE XII.—A. B., aged seven years. Admitted with faucial diphtheria.

Nose: No exudate; no discharge; no crusts.

Throat: Tonsils are enlarged; upper faucial structures are infiltrated; thick, soft, smooth, white exudate is spread over tonsils, anterior pillars, and greater part of uvula, extending well forward on the soft palate. A moderate congestion; no punctation.

The child very much prostrated.

On June 9 child developed a syrupy nasal discharge, which was probably an extension of the diphtheritic process up the postnasal fossæ.

On the 15th developed a soft, musical murmur at the apex, systolic in time, accompanied by slight cardiac dilatation.

On June 8 showed albuminuria and the presence of epithelial, granular, and hyaline casts.

Culture: Positive.

Result: Discharged, cured, June 23, 1906.

(a) June 7, 1906, 6.40 P.M., fifth day of disease, 16,800 leukocytes:

Polymorphonuclears	74.0 per cent.	12,432.0 per c.mm.
Small lymphocytes	16.4 "	2,755.2 "
Large lymphocytes	8.8 "	1,478.4 "
Eosinophiles	0.6 "	100.8 "
Basophiles	0.2 "	33.6 "

(b) June 7, 1906, 10.10 P.M., 18,800 leukocytes. Two hours and thirty-five minutes after first dose of 7500 units:

Polymorphonuclears	73.2 per cent.	13,761.6 per c.mm.
Small lymphocytes	16.0 "	3,008.0 "
Large lymphocytes	9.8 "	1,468.4 "
Eosinophiles	0.4 "	75.2 "
Basophiles	0.6 "	112.8 "

(c) June 10, 1906, 11.30 A.M., eighth day of disease, 15,800 leukocytes. Twenty-four hours after last dose, in all, 22,500 units:

Polymorphonuclears	76.6 per cent.	12,102.8 per c.mm.
Small lymphocytes	12.4 "	1,959.2 "
Large lymphocytes	8.2 "	1,295.6 "
Eosinophiles	2.2 "	347.6 "
Basophiles	0.6 "	94.8 "

CASE XIII.—J. P., aged twenty-two months. Admitted with tonsillar and laryngeal diphtheria.

Nose: Slight mucoid discharge; no exudate; no crusts.

Throat: Both tonsils are covered with thin, rough, soft, gray exudate; slight congestion; no punctation.

Was croupy at the time of admission and was intubated about three hours afterward.

On June 14 developed a small area of bronchopneumonia; was extubated on the eighth day and remained without tube thereafter.

On the 15th showed presence of albumin in the urine and a few hyaline casts.

Culture: Positive.

Result: Discharged, cured, July 7, 1906.

(a) June 13, 1906, 1.45 P.M., fifth day of disease, 14,700 leukocytes:

Polymorphonuclears	71.0 per cent.	10,437.0 per c.mm.
Small lymphocytes	14.0 "	2,058.0 "
Large lymphocytes	11.8 "	1,734.6 "
Eosinophiles	3.2 "	470.4 "

(b) June 13, 1906, 11.10 P.M., 10,000 leukocytes. Nine hours and ten minutes after first dose of 10,000 units:

Polymorphonuclears	69.2 per cent.	6920.0 per c.mm.
Small lymphocytes	18.6 "	1860.0 "
Large lymphocytes	11.8 "	1180.0 "
Eosinophiles	0.4 "	40.0 "

(c) June 16, 1906, 2.15 P.M., eighth day of disease, 19,800 leukocytes. Twenty-seven hours after last dose, in all, 30,000 units:

Polymorphonuclears	76.8 per cent.	15,206.4 per c.mm.
Small lymphocytes	12.4 "	2,455.2 "
Large lymphocytes	8.8 "	1,742.4 "
Eosinophiles	2.0 "	396.0 "

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February, 1908.

Tissue Transplantation into Different Species.

BY LEO LOEB AND W. H. F. ADDISON.

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University of Pennsylvania.)

THE following investigations represent one part of a connected series of researches into the condition of tissue growth.¹ Former studies demonstrated the importance of the character of the body fluids for the life and growth of transplanted tissues.² We expected that a systematic investigation into the transplantation of one kind of tissue of a certain species into a variety of other animal species might throw additional light on the problems of tissue growth. We used for this purpose the skin of the guinea-pig, and carried out series of transplantations into the guinea-pig, the rabbit, dog, pigeon, and frog. Pigmented skin of the guinea-pig ear was used in all cases; it was first thoroughly washed with soap and water, then cleaned with

¹ Leo Loeb, Beiträge zur Analyse Gewebewachstums I, Arch. f. Entwicklungsmechanik, 1907, vol. xxiv.

² Leo Loeb and Samuel Leopold, on the Difference in the Results Obtained after Inoculation of Tumors into the Individual in which the Tumors had Developed, etc., Jour. of Med. Research, vol. xvii, No. 3.

alcohol and bichloride of mercury. The skin was transplanted into pockets in the subcutaneous tissue of the various animals. The pieces were removed after various periods and most of them cut into serial sections.

We know of only two investigators who transplanted skin into animals of different species. Beresowsky¹ covered defects of the skin of dogs with flaps of frog skin; the transplanted frog skin became necrotic, was infiltrated by leukocytes, and cast off. A similar fate befell pieces of dog skin transplanted upon wounds in the guinea-pig. Here the skin remained preserved somewhat longer, but at no time were mitoses or any progressive changes noticeable. The technique in the experiments of Beresowsky differed somewhat from our own. H. Ribbert,² on the other hand, used the technique employed in our investigations. This author transplanted pieces of guinea-pig skin and human skin into the subcutaneous tissue of the rabbit. He summarizes his results as follows:

In the first three days proliferation of the transplanted epithelium takes place. The epithelium grows partly upon the connective tissue of the rabbit and of the guinea-pig. All these proliferative phenomena, however, are not as marked as after transplantation into the same species. After three days the process of growth ceases. The layers of epithelium are still preserved on the fourth and fifth day; but at this period the nuclei no longer stain well, and the cells swell. On the eighth day the pieces are seen degenerating. His explanation of these phenomena is as follows:

In the beginning the cells have nourishment which is sufficient for proliferation. The host supplies merely indifferent food substances, as oxygen and water. The transplanted cells cannot use the food substances of the host, therefore they all die after some time. We notice that Ribbert does not differentiate between the behavior of the skin of the guinea-pig and that of the human being when transplanted into the rabbit. From his brief description we may judge that he bases his conclusions mainly on the study of the guinea-pig skin

¹ Ueber d. Histolog. Vorgänge bei der Transplantation von Hautstücken auf Thiere einer anderen Species, Ziegler's Beitrage, 1893, Band xii.

² Ueber Transplantation auf Individuen anderer Gattung. Verhandl. d. pathol. Gesellschaft, Breslau, 1904.

transplanted into the rabbit. We aimed, on the other hand, especially at comparing the results of the transplantation of guinea-pig skin into species nearly related to the guinea-pig, and into others farther removed, as the pigeon and the frog. We carried out experiments as shown in the accompanying table:

TABLE OF TRANSPLANTATION OF GUINEA-PIG SKIN INTO DIFFERENT SPECIES.

		Days									
		1	2	3	4	5	6	7	8	9	10
Guinea-pig	(a)	10	8	8	5	16	4	7	5	..	7
	(b)	9	8	7	5	13	4	7	5	..	6
	(c)	9	8	5	5	11	4	6	5	..	4
		(2 m.)	(6 m.)	(5 m.)	(5 m.)	(10 m.)	(1 m.)				
Rabbit	(a)	2	12	14	4	10	3	7	2	..	8
	(b)	1	6	8	..	2	..	3	2	..	2
	(c)	..	2	5	..	1	..	2	1		
				(3 m.)		(1 m.)		(2 m.)	(1 m.)		
Dog	(a)	6	9	10	4	18	6	8	8
	(b)	1	6	3	3	6	4	1			
	(c)	..	2	1	1	3	2	1			
			(2 m.)	(1 m.)	(1 m.)	(3 m.)	(2 m.)	(1 m.)			
Pigeon	(a)	4	7	13	10	16	..	10	..	1	15
	(b)	4	7	7	6	10	..	2	2
	(c)	1	4	6	5	5					
			(2 m.)	(5 m.)	(3 m.)	(4 m.)					
Frog	(a)	6	1	3							
	(b)	1	0	0							
	(c)	0	0	0							

(a) Shows number of pieces transplanted for each period.

(b) Shows number of pieces which have living epithelium.

(c) Shows number of pieces which have large amount of living epithelium or mitoses. The number in brackets shows in how many of these pieces mitoses have been observed.

SUMMARY.

1. After transplantation of guinea-pig skin into animals of another species the epithelium does at no time grow as actively as after transplantation into the guinea-pig.

2. The period of active proliferation as evidenced by the presence of mitoses is considerably greater than found by Ribbert. Instead

of three days, we found growth taking place in the guinea-pig skin, in the rabbit as late as eight days, in the dog seven days, and in the pigeon five days after transplantation.

3. If we consider, however, the different series of transplantations as a whole, the difference between the results of transplantations into the guinea-pig, on the one hand, and into other species, on the other hand, becomes more marked at later periods after transplantation, no active proliferation having been noticed in any animal except the guinea-pig later than eight days.

4. There is a distinct difference in the energy of growth of transplanted pieces according to the species into which the guinea-pig skin has been transplanted. The proliferative energy manifests itself longest in the rabbit, namely, eight days. Not quite so favorable as the rabbit is the dog, in which the growth ceases after seven days. In the pigeon no growth takes place after five days. In the frog no growth takes place at any time, as might be expected if we consider the body temperature of this animal, which is not sufficiently high to permit growth phenomena in mammalian tissues. But the conclusion that the frog is a very unfavorable soil for the transplanted guinea-pig skin is also suggested by the results of the re-transplantation of the guinea-pig skin from the frog into the guinea-pig, inasmuch as pieces which had been kept longer than three and a half hours in the frog did, in no case, grow after re-transplantation into the guinea-pig, and in these the growth was very insignificant.

5. The differences between the growth of guinea-pig skin transplanted into the rabbit, on the one hand, and into the pigeon, on the other hand, would, in all probability, have been still more striking but for the interference of another factor, that of bacterial infection. Bacteria which cannot be entirely eliminated from the guinea-pig skin cause least interference with the growth of the epithelium after transplantation into the guinea-pig, nor do they become very active in the pigeon. But they are a factor seriously interfering with the result of the transplantation into the rabbit and also into the dog. The collections of leukocytes found in the different species after transplantation of the skin can probably be used as an indicator of the growth and toxic action of bacteria transferred with the skin. In many cases the negative results after transplantation of skin into

rabbit and dog can be brought into causative relation with the presence of bacteria as indicated by leukocytic infiltration. The small importance of this complicating factor in the pigeon causes the skin of the guinea-pig to be under relatively much more favorable conditions in the pigeon, and permits comparatively many pieces of skin to grow in the first five days. Since, notwithstanding the absence of infection, the pieces suddenly cease to grow in the pigeon after five days, although they continue to grow for a longer period in a certain number of cases in the dog and in the rabbit, we may conclude that the body fluids of the pigeon are more injurious to the guinea-pig skin than those of the rabbit and of the dog.

6. Although collections of leukocytes are almost absent around the transplanted guinea-pig skin in the frog, they appear usually very abundantly after re-transplantation of the guinea-pig skin into the guinea-pig. This fact indicates an increased growth or an increased virulence of the bacteria after re-transplantation into the guinea-pig. Notwithstanding the existence of this complicating factor, the fact that after a presence of more than three and a half hours in the frog, the guinea-pig skin has, after re-transplantation into the guinea-pig, lost its entire proliferative energy, renders it very probable that the body fluids of the frog have a direct injurious influence on the epithelium of the guinea-pig.

7. From these experiments and considerations the conclusion seems justifiable that there exists a difference between the adequacy of different species as a soil for epithelium of the guinea-pig, and that the more nearly related two species are, the better will be the growth of the transplanted epithelium. We may, therefore, arrange the different species in the following order, indicating the gradual decrease in the adequacy of the soil for transplanted guinea-pig skin: (1) Guinea-pig; (2) rabbit; (3) dog; (4) pigeon; (5) frog.

8. If we leave out of consideration the factor of bacterial activity, which causes the necrosis of a relatively large number of pieces of skin, we observe the following processes leading to the death or disappearance of the transplanted pieces.

(a) The signs of active proliferation cease, the cells remain alive for some time, and in certain instances active keratinization takes place. They are finally destroyed under the influence of the ingrowing connective tissue.

(b) The cells still show signs of proliferation, but the growing connective tissue of the host surrounds more and more the transplanted piece, and begins to press upon it. Small round cells, migrating from the connective tissue of the host, invade the transplanted epithelium. The transplanted epithelium cannot resist the pressure and invasion for any length of time, and will gradually disappear. One of us¹ described similar phenomena in the guinea-pig in cases in which, especially after consecutive serial re-transplantations of the same piece of skin, the epithelium was unable to form a cyst. Under such conditions the epithelium will even disappear in the guinea-pig. After transplantation into individuals of another species the cell growth is in no case sufficiently strong to permit the cells to close into a cyst. They will, therefore, in each case die ultimately under the influence of the surrounding host connective tissue, even if the pieces have not become necrotic at an earlier period.

(c) It is very likely that a certain number of pieces become directly necrotic under the influence of the body fluids of animals of another species, although we cannot with certainty exclude the possibility that bacterial toxins are, in most cases, responsible for this kind of cell death.

There is one point which deserves still an especial mention, that is, the different degrees of liability of infection which we find in different species of animals toward the tissue of a certain species. These findings are not accidental, not due to mistakes of technique, but are evidently caused by a determined relation between certain organisms found on the skin, and certain species of animals. It is intended to study these relations in later investigations.

As to the theoretical interpretation of these results, and as to their bearing upon the problems of tissue growth, it is quite certain that the interpretation of Ribbert is inadequate. He assumes that the cells live on their stored up food material and are unable to assimilate the food of the host. Such a food was, afterward, designated by Ehrlich as X substance, and an increased avidity for this substance was declared by this author to distinguish tumor cells from ordinary tissue cells. Because the foodstuffs of different species differ, tissues

¹ Beiträge zur Analyse d. Gewebewachstums I, Archiv f. Entwicklungsmechanik, 1907, vol. xxiv.

transplanted into individuals of another species can, therefore, live and grow only until their own stored-up food-substance has been used. Such an explanation might have been found satisfactory, if it had been shown that the tissues behave alike in individuals of different species, without consideration as to whether the species of the animal from which the piece was taken and the species of the individual into which it was transplanted were nearly related or were very distant. That such a relationship exists is, however, made very probable through our experiments. Such an hypothesis is furthermore not able to explain a fact emphasized by one of us in former publications,¹ namely, that there exist even differences in the life and growth of tissues after transplantation into the individual in which the tissue had taken its origin, and into other individuals of the same species.

In order to explain these facts we have to assume either (1) the existence of a number of growth substances necessary for the full development of the tissues—these would be partially present in other individuals of the same species, present in smaller number in individuals of a different species, and absent in individuals of distant classes of animals—or we have to assume (2) that the more distantly two species are related, the more substances are present which inhibit the growth of the tissues of other species. That directly injurious substances are active even after transplantation in the same species seems to be indicated by the fact that even after transplantation of skin into an individual of the same species the hair follicles are frequently the only tissues preserved and, that they are the favorite seat for mitoses to occur. Surrounded on most sides by connective tissue, the hair follicles seem to be less accessible to the injurious influence of certain substances after transplantation. Injurious agencies of a chemical or physico-chemical character seem to be especially active in certain species. We noticed, for instance, especially after transplantation into the pigeon, that in the course of the first few days a swelling of the nuclei and cytoplasm, with consecutive washing out of the cytoplasm and karyolysis, is found to occur. In other species this change was much less frequent.

¹ Leo Loeb, Further Investigations in Transplantation of Tumors, *Jour. of Med. Research*, 1902, vol. viii. Leo Loeb and Samuel Leopold, On the Difference in the Results Obtained after Inoculation of Tumors into the Individual in which the Tumors had Developed, etc., *Jour. of Med. Research*, xvii, No. 3.

Whatever might be the ultimate conclusion as to the presence of injurious substances or to the absence of the necessary growth substances in more distant species, or as to the importance of both of these factors, we certainly find, here, indications of a specific physico-chemical adaptation between cells and body fluids in one and the same species, or even in one and the same individual. An analogous specific adaptation has been shown by one of us to exist between certain cell constituents, namely the tissue coagulins and the fibrinogen, a constituent of the blood plasma.¹

From the growth substances referred to here, which are active in individuals at all times, we have to distinguish certain temporarily active or intermittent growth substances, such as the ones secreted at certain periods by the ovaries, causing the growth of decidual new formations,² or substances which are active at later periods of pregnancy, causing the growth of the mammary gland and of certain mammary tumors.³

¹ Leo Loeb. Versuche ueber einige Bedingungen d. Blutgerinnung, insbesondere d. Specificität, etc., Vichow's Arch., 1904, Band clxxvi.

² Ueber d. exper. Erzeugung von Knoten von Deciduagewebe, Centralblatt f. allgem. Pathologie, 1907, Band xviii.

³ Further Investigations in Transplantations of Tumors, Jour. of Med. Research, 1902, vol. viii.

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The Theory of Chemical Correlation as Applied to the Pathology of the Kidney.¹

BY RICHARD M. PEARCE, M.D.,
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WHEN invited by your committee to deliver this address, an honor which I assure you I highly appreciate, it seemed to me most appropriate that I present to you, as members of a society which has so wisely fostered the application of pathology to clinical medicine, a subject having also this twofold interest.

There is, perhaps, no problem in medicine of greater interest to clinician and pathologist alike than that of the relation which some of the more important manifestations of chronic nephritis, as vascular hypertension, heart hypertrophy, uremia, and edema, bear to the renal lesion itself. During the past summer, having in view an experimental study of some phases of vascular hypertension, I undertook a critical

¹ Address at the Annual Conversational Meeting.

review of the general literature of renal physiology and pathology, and was amazed, not only at the multiplicity of theories concerning the relation of the kidney to heart hypertrophy and hypertension, but also at the importance attached by many writers to the part played by the hypothetical internal secretion of the kidney. I use the term "hypothetical" advisedly, for although certain clinical observations are very suggestive, the experimental basis upon which the theory rests appeared to me to be most unstable. Further study of the subject, however, led to the conclusion that the theory, despite conflicting observations, offered, in view of our newer theories of chemical correlation, much that was suggestive, and, indeed, if established by proper experimental evidence, of fundamental importance in the pathology of the kidney. At the same time I felt that if it were not capable of proof it should be discarded as a hindrance to more profitable study along other lines.

For these reasons I have for the past six months devoted my time entirely to various phases of this problem, and have attempted to determine also the effect of products retained in, or arising within, the organism during nephritis, and of the correlation of disease of the kidney with the internal secretion of other organs, as, for example, the adrenal; in short, a study of chemical correlation in its broadest sense as applied to the kidney. It is the results of this study I present this evening. Such an investigation, as it naturally includes to a considerable extent a repetition of the experiments of others, and as its scope is somewhat comprehensive, is as yet far from complete. It has advanced sufficiently, however, along all lines to allow me to give you a more or less satisfactory summary of my results.

I wish at the outset to have it clearly understood that I use the term "chemical correlation" in the broadest possible sense to include the action on any organ or tissue, or group of organs or tissues, of any substance or substances present in the blood as the result of the normal or abnormal life processes of any other organ or tissue. My discussion must therefore include the older theory of internal secretion with its newer aspect, the action of hormones, as well as the action of products of metabolism. I do not think it necessary to explain these theories to this audience. As examples of these types, respectively, it is suffi-

cient to mention the secretion of the adrenal, the action of "secretin," and the influence of carbon dioxide on the respiratory centre.

The knowledge derived from these various phases of chemical correlation will be applied to the pathology of the kidney in an attempt to explain, or at least offer a working basis for, the understanding of the increased blood pressure, the uremia, the edema, and the disturbance of metabolism associated with chronic renal disease. And although I have been unable to reach definite conclusions in regard to all these matters, I think a presentation from this point of view may perhaps be suggestive and an incentive to discussion.

With this explanation we may glance for a moment at the older observations upon which the theory of internal secretion, as applied to the kidney, is based. The subject was first brought to general notice by Brown-Séquard, who, as the result of his investigations of the internal secretion of the testicle, came to very broad conclusions concerning internal secretions in general, and stated that the kidney, as other organs, has this function. He based his opinion as regards the kidney on clinical observation and on the study of dogs from which both kidneys had been removed, as compared with those in which the ureters had been ligated. As the result of both procedures the retention of metabolites is the same, but in the nephrectomized animals, death, which comes on more rapidly, is supposed to be due to the absence of an internal secretion which, it is assumed, is still furnished by the ligated kidneys. If, he claimed, in the nephrectomized animals the internal secretion is replaced by the injection of renal juice, or glycerin extracts of kidney, or by normal serum, the animals live as long or even longer than those which have had both ureters ligated. Such observations are urged in support of the theory of internal secretion by those who do not believe uremia to be due to the retention of the products of metabolism.

Bradford's extirpation experiments, characterized by a marked wasting of the body, polyuria, and increased loss of nitrogen, with especially an increased elimination of urea, are also offered in support of this theory, although Bradford states definitely that he made no observations which indicated that these changes were due to the disturbance of an internal secretion.

Similarly metabolism experiments on man which tend to show that uremia may occur without evident nitrogen retention are viewed in the same way and are taken by those who support the theory of internal secretion to indicate that uremia is not due to lack of elimination of metabolites, but to loss of the internal secretion.

Analogous to the injection of kidney extracts into nephrectomized animals are the attempts at organotherapy—the feeding of kidney extracts to nephritics with or without uremia—having for their object the replenishment of the internal secretion which the diseased kidney fails to furnish.

As regards the effect of the kidney on blood pressure, Tigerstedt and Bergman found that extracts of the kidney substance of the rabbit prepared in various ways caused a rise in pressure when injected into the rabbit. These experiments are frequently quoted in the discussion of the hypertension of contracted kidney as evidence of the effect of an internal secretion on the vascular system. To these older views we may add more recent observations, such as those which assume a correlation between the adrenal and the kidney of chronic interstitial nephritis in the production of arteriosclerosis and the question of the development of endotheliotoxic and nephrotoxic substances during nephritis.

All these observations, which must be considered in connection with the theories of internal secretion and chemical correlation, may be grouped for purposes of discussion under the following headings:

1. Clinical observation of the effects of nephrectomy and ligation of the ureter in man.
2. Experimental study of similar conditions in animals.
3. Therapeutic experiments on nephrectomized animals under above conditions.
4. Therapeutic experiments with kidney extracts on nephritics with or without uremia.
5. Metabolism studies in chronic nephritis in man.
6. Metabolism studies in animals with partial extirpation of kidney.
7. Experimental study of the effect of kidney extracts on the blood pressure.

8. The effect on the heart and blood pressure of the removal of large portions of the kidney.

9. The effect on the blood pressure of the serum of animals with experimental nephritis.

10. The relation of chronic interstitial nephritis to increased secretion of the adrenal and the influence of the latter upon the cardiovascular system.

11. The determination, by physiological methods, of the presence of adrenalin or adrenalin-like substances in the serum of nephritics or of animals with lesions of the kidney.

12. The study of nephrotoxic and endotheliotoxic substances in the serum of animals with experimental nephritis.

The first five of these may be disposed of briefly:

1. *Clinical Observations.* These offer the most suggestive evidence of some influence which prevents the appearance in individuals with obstruction, or accidental ligation of both ureters, of the uremia which so early appears after complete nephrectomy. For example, in 66 per cent. of Fowler's series of 93 cases of ureter obstruction no marked uremic symptoms were evident. In such cases death is usually delayed for two weeks or more, with few or no symptoms of uremia, while after nephrectomy it occurs usually in four to six days, and commonly with well-marked uremic manifestations. That this difference may be due to an internal secretion is possible, but chemical or mechanical factors must be considered. It is known that the kidney has the power to form from substances brought to it in the blood certain new compounds, as for example, hippuric acid from aromatic derivatives and glycocholic acid from glycine. It is possible that it has other synthetic or analytic functions, or perhaps a detoxicating power similar to that of the liver. Under such circumstances even though the ureter be ligated, these functions would persist and control to a certain extent an auto-intoxication. It has been shown also, as the result of experimental studies, that urine is excreted until such time as the pressure in the dilated pelvis equals that of the blood. The dilatation of the pelvis under these circumstances may become very great and a considerable quantity of fluid is contained therein, and it is possible that an exchange of fluids between the pelvis and

surrounding tissue may take place, and thus by allowing elimination of toxic substances through other channels, as skin, intestine, or lungs, delay the symptoms which appear so rapidly after nephrectomy, a procedure which necessarily means an immediate and complete retention in the blood of the products of catabolism. Until the influence of such factors and also of the nervous system is settled experimentally we can merely look upon clinical observations as suggestive of internal secretion but capable of interpretation in other ways.

2. *Experimental Nephrectomy and Ureter Ligation in Animals.* In animals the recognition of symptoms which may be considered uremic is exceedingly difficult and practically impossible, and the only criterion available is the difference in time which elapses before death in ligation on the one hand and nephrectomy on the other. Such observations naturally receive the same interpretation as clinical experience.

3. *Therapeutic Experiments on Animals.* Brown-Séquard and others, but more especially his pupils, have attempted to show that the life of the nephrectomized animal may be prolonged and uremia prevented by injecting extracts of kidney substance or the serum or defibrinated blood of normal animals, the blood being taken from the renal vein or sometimes indeed from the general circulation. These are not conclusive. The difference between the behavior of control and treated animals is but slight, and the period of survival varies greatly in the control. For example, in the experiments of Ajello and Parascandalo twelve control dogs died in from four to forty-eight hours, with dyspnea, convulsions, and gastro-intestinal disturbances considered to be uremic in character; while of ten treated with glycerine extracts, one lived four days, six three days, and the other three died in from forty-eight to fifty-two hours. Many other investigations, mainly of French and Italian workers, give similar results. Usually quoted in this connection is the work of Meyer, a pupil of Brown-Séquard, who considered the dyspnea of nephrectomized animals, which he describes as of a Cheyne-Stokes variety, to be the best criterion of the condition of uremia. Recording the respiration graphically, he took the momentary cessation of dyspnea resulting from the intraperitoneal injection of kidney extract, or the intra-arterial

injection of defibrinated blood of the renal vein, as a sign of improvement. His conclusions have been severely criticised on account of the ease with which, in such experiments, disturbances of respiration occur, the misleading nature of the method of recording, and the influence of removing large quantities (60 c.c.) of blood from the circulation before injecting equal amounts.

The work on this subject shows that animals receiving extracts of the kidney or defibrinated blood of the renal vein certainly do appear to have a longer postoperative life, but the difference is slight and is not evident when the blood serum of the renal vein, which would be supposed to contain the internal secretion, is used, as in the experiments of Chatin and Guinard. From these latter experiments one is led to conclude that if the serum of the renal vein is without effect, then either the internal secretion is carried by the red corpuscles or the corpuscles in some way aid in prolonging the postoperative life of the nephrectomized animal. From a survey of the literature one gathers the impression that these therapeutic experiments are of no value as support of the theory of internal secretion.

4. *Therapeutic Experiments on Man.* These need detain us but a moment. First attempted by Dieulafoy, who recommended the subcutaneous injection of glycerine extracts of the kidney of the guinea-pig and the ox under the name of "nephrene," this method was exploited by many in the treatment of nephritis and especially of uremic crises. Other preparations of kidney, fluid and dry, and even injections of the blood of the renal vein were employed, but the favorable results at first reported were not constant and soon were regarded as coincident with the periods of temporary improvement so frequent in nephritis. Renal opotherapy has apparently been almost entirely abandoned. In Kaufmann's summary (1905) of the literature of the subject there are few references to its use in recent years. With its disappearance as a therapeutic measure disappears also one of the arguments in favor of the internal secretion of the kidney.

Somewhat different from opotherapy are the recent experiments of Carnot and Lélièvre, who have injected subcutaneously or given by mouth the serum and kidney extracts of animals which have withstood a unilateral nephrectomy. They claim that such treatment

stimulates the regeneration and growth of kidney parenchyma even to the extent of the formation of new glomeruli and tubules. This influence they term nephropoietic.

5. *Metabolism Studies on Man.* The occurrence of uremia without nitrogen retention and, on the other hand, the absence of uremia with decided nitrogen retention has led to the suggestion that uremia might be due to a gradual diminution of the internal secretion of the kidney rather than to a retention of the products of metabolism. Owing to our lack of knowledge of the real nature of uremia this view, which certainly is suggestive, has some support.

It is well known as pointed out by von Noorden that in non-uremic nephritics the protein catabolism follows the same course as in health. If such individuals be given a diet which in a normal individual would establish nitrogenous equilibrium, either of the following results may occur:

1. The excretion of nitrogen through the kidneys and in the feces will correspond to the quantity introduced. This normal condition may occur especially in renal cirrhosis.

2. The quantity of nitrogen excreted may be markedly less than that ingested. This may be explained in part by increased elimination by the lungs, intestine, or skin, but is also, in part, a nitrogen retention.

3. More nitrogen may be present in the urine than has been ingested. This is due to an increased permeability of the kidney and is analogous to the increased elimination of nitrogen resulting from the administration of large quantities of water.

These conditions may occur in any of the types of renal disease, and may alternate in a single individual. As emphasized by von Noorden, the second type, or that of retention, in renal cirrhosis is seldom permanent, for it is succeeded either gradually or suddenly by one in which nitrogen is freely excreted and which restores the general daily average of nitrogen elimination.

On the other hand, almost all observations regarding the metabolism of patients suffering from uremia show a more or less considerable retention of nitrogen. The cases of uremia with excellent elimination of nitrogen are exceptions and, as von Noorden points

out, recall "an old remark of Bortels that uremic convulsions sometimes occur when edema is already diminishing—at a time, that is, when nitrogenous extractives return from the tissues into the blood in large amounts, and so come in contact with the nervous system."

It would appear, therefore, that the detailed study of the metabolism of nephritis does offer a possible explanation of uremia in the absence of nitrogen retention without the aid of the theory of internal secretion.

6. *Metabolism in Extirpation Experiments on Animals.* From the experimental side we have the observations of Bradford and of Bainbridge and Beddard. The former, working with dogs, found that after the removal of approximately three-fourths of the total kidney weight death occurred in from one to six weeks from asthenia and with great wasting; coma and convulsions were not observed. Death is apparently dependent on the amount of kidney substance removed, and not upon mutilation inflicted by the operative procedure. Excision of a portion of one kidney or portions of both is followed by an increase in the volume of the urine, but unaccompanied by an increase in the total solids. The latter does occur, however, after excision of three-fourths of the total kidney weight. This increase is absolute when appetite does not fail, and relative when little or no food is taken. Under the latter circumstances the amount of urea eliminated is as great as that excreted previously on a full diet. At the same time the blood and tissues, particularly the muscles, show a considerable increase in nitrogenous extractives. Bradford concludes that the disturbance of metabolism following the reduction of kidney substance is due not to the retention of the products of normal destruction of tissue, but to an increased tissue catabolism, especially of the muscles, producing large quantities of urea. He states that he has made no observations to determine whether this is due to the cessation of the action of a renal internal secretion. His results have, however, been so interpreted by later writers.

Bainbridge and Beddard in a recent publication describe their observations on cats. They conclude that the removal of three-fourths of the kidney substance causes loss of appetite, wasting, and death in a few days or weeks; that an increase of nitrogen in the urine is not constant, and occurs only during the last few days of life when

the animal has lost 22 per cent. or more of its body weight, and therefore that the kidney has no direct influence on metabolism, but that the increase of nitrogen is the result of inanition and similar to that which occurs in starving animals. They also found that there was not necessarily an increase in the volume of urine.

The difference of opinion between these investigators seemed sufficient to warrant a repetition of the experiments in order to determine the effect of the reduction of the kidney on nitrogenous metabolism. In carrying out the same I have also investigated the feces in order to determine if possible whether or not the inanition could be explained by digestive disturbances due to faulty absorption, or possibly to the effect of irritating substances eliminated through the intestines as the result of faulty chemical correlation.

These experiments were made upon dogs which were kept for some time before operation and during the entire course of the experiment, in nitrogenous equilibrium. For this purpose a purin-free diet of casein, cracker-dust, and lard was used and the amount of water limited to 600 c.c. The animals were kept in the usual well-ventilated metabolism cages and were catheterized at the end of each twenty-four hours. After each catheterization the bladder was washed out and the wash water added to the catheterized urine and that voided naturally and the whole made up to a definite volume. This urine was carefully preserved from changes of any kind until the analyses were made. When albuminuria occurred, which occasionally happened during a short period immediately following the operation, the coagulable protein was removed by heat and acetic acid, the coagula being thoroughly boiled out with water and the washings added to the urine.

Upon these twenty-four-hour samples the following determinations were made: Total nitrogen by the Kjeldahl method; ammonia by the Shaffer method, and urea by the Morner Sjoquist method. The investigation was so limited mainly because the changes in total nitrogen and in the elimination of urea and ammonia are the only points in dispute and in part on account of the negative nature of the results obtained by Bradford and by Bainbridge and Beddard in regard to other substances.

The general procedure was to place the animal in nitrogenous equilibrium, conduct control determinations for a period of three days, operate, and after allowing two or three days for recovery from the acute effects of the operation, make determinations during one or more three-day periods. The operative procedure differed somewhat from that of the English investigators. Instead of removing a wedge of kidney substance, the upper half of the kidney was removed and the bleeding from the cut surface controlled by a mattress suture. This method, although it narrows the pelvis somewhat, is not followed by extensive infarction or hemorrhage and gives better results than that recommended by Bradford. At a subsequent operation either one-half the opposite kidney or the entire kidney was extirpated, and in the case of the former the remaining half was taken out at a third operation. In some instances one entire kidney and half the opposite kidney were removed at one operation without any immediate ill effect. The present work includes metabolism studies on but four dogs with varying degrees of kidney reduction, but a somewhat exhaustive study of the general effects of extirpation and of the process of repair in the kidney after various forms of operative injury will be presented later by Dr. J. A. Sampson and myself.¹

At present it is sufficient to state that we have had no difficulty in keeping animals alive and in good condition with neither general nor local disturbances after the removal of one-quarter, one-half, or, in some instances, three-quarters of the total kidney substance at one operation. The removal of larger amounts and occasionally of three-quarters, is followed by severe general disturbances, which have rendered futile all attempts to maintain the animal in nitrogenous equilibrium. In one instance, after removal of a considerable portion of the kidney substance, a nephritis developed which added to the value of the experiment rather than otherwise.

The results² in the four experiments thus far completed may be summarized as follows:

Experiment I shows no appreciable changes in metabolism after the

¹ Journal of Experimental Medicine, 1908, No. 6.

² For the details of these experiments, see Journal of Experimental Medicine, 1908, No. 5.

removal of one-half of one kidney or one-half of each kidney. This animal unfortunately died a few hours after the third operation with no lesions discoverable at autopsy except a very extensive edema of the lungs. Experiment II indicates that the removal of two-thirds of the entire kidney substance at one operation does not interfere with the general condition of the animal, as shown by the constant weight and normal metabolism figures.

The third experiment on an animal with a spontaneous nephritis, a condition occasionally found in stray dogs, shows that the lesion has no effect on metabolism, even after the kidney substance is reduced one-half by a unilateral nephrectomy.

The fourth experiment was not planned for a study of metabolism, but when it was found that an animal with but one-quarter of its kidney substance had developed an acute nephritis, thus diminishing, it was assumed, the functional capacity of the fractional portion of the kidney remaining, it seemed too good an opportunity to lose, and the animal was placed on a constant diet and the metabolism experiments carried out six days later. This animal, despite the great reduction of kidney substance and the presence of a nephritis, had normal metabolism during the first three-day observation period. Two weeks later, however, when its appetite had begun to fail, equilibrium was lost, although no change in the percentage relations of nitrogen was evident.

But after another period of two weeks had elapsed, and the kidney substance had still further been reduced by operation, leaving the animal with but one-seventh of its original kidney weight, a change in the urea-ammonia ratio, indicative of inanition, occurred. That this final change is due, as Bainbridge and Beddard claim, to inanition there can be no doubt. Up to this point, the beginning of starvation, the changes in urea described by Bradford were not observed, and there is no evidence that the kidney, through an internal secretion or otherwise, has any influence on general nitrogenous metabolism, and I believe the theory of internal secretion, so far as it concerns general metabolism at least, may be set aside.

There remains, however, the very important question of why the removal of more than three-fourths of the kidney substance leads to

loss of appetite and consequent inanition. Although occasional vomiting occurred in these animals it did not seem to be sufficiently frequent or severe to indicate a general gastro-intestinal disturbance. To test this point extirpation of the kidney was done on two dogs after the establishment of a gastric fistula. In this way it was hoped that food necessary for nitrogenous equilibrium could be introduced artificially, and by the examination of both urine and feces some light might be thrown on the cause of the disturbance. These efforts were rendered futile, however, by the inability of the stomach to retain the materials introduced. The conclusion is inevitable, therefore, that although the kidney appears to have no direct influence on nitrogenous metabolism, the removal of large portions of its substance does indirectly lead to disturbances of general nutrition by interfering with the functions of the alimentary canal.

Examination of the Feces. The occurrence of these serious gastric and intestinal disturbances in animals after kidney reduction, and their general similarity to lesions occurring occasionally in man in the course of chronic nephritis, led me, in view of the more recent ideas of the chemical control of the body and the influence of one secretion on another, so well brought out by Starling's investigations, to study the feces of these animals in order to determine if partial nephrectomy had any influence on digestion, absorption, or elimination into the intestine. Bainbridge and Beddard's claim that the disturbances in metabolism are due to inanition dependent on vomiting and diarrhea, with eventual failure to take food, and not to a direct influence of the kidney on metabolism, dodges the question. The point to be determined is whether or not these gastric and intestinal disturbances are caused by faulty absorption or by digestive disturbances due to the elimination into the intestine of substances normally removed by the kidneys. The demonstration of such a relation would be important not only as an illustration of the chemical interrelationship between various organs, but would also aid in explaining similar disturbances associated with the nephritis of man.

These latter, which, I understand, are frequently so severe as to present symptoms closely resembling a violent gastro-enteritis, have been ascribed in part to alterations of the mucosa due to edema, and

in part to the influence of the uremic condition on the central nervous system. While these explanations account for many of the symptoms, others, according to von Noorden, must be attributed to toxic chemical action. In fact, recent investigations show that in uremia substances usually eliminated by the kidneys are secreted vicariously into the alimentary tract. Of these the most irritating chemically is ammonia, which is formed in the intestine by decomposition of the secreted urea. The feces of uremic diarrhea have been found to be extremely rich in ammonia.

Studies of the feces having for their object the determination of the degree of absorption in nephritis, for which we are indebted mainly to von Noorden, show that the absorption of fats is very complete. The situation in regard to nitrogen is not so clear, the loss in some cases being greater than normal, while in others an abnormally high percentage is found. The variation in some individuals, without a corresponding change in diet or in the nature of the stools, or in the general condition, and with no change in the percentage of dry substance or fat content of the feces, led von Noorden to the conclusion that the increase of nitrogen was due not to impaired absorption, but to the vicarious excretion of metabolites stored up in the organism. In many cases the excretion of nitrogen remains normal. High amounts, above three grams daily, are found in nephritis only in uremic diarrhea, and are due largely to a high content of ammonium salts, constituting sometimes 10 to 20 per cent. of the total fecal nitrogen.

In the four experiments summarized above, the total nitrogen in the feces evacuated during control periods and periods of metabolism study was estimated. The results indicate no marked change in the total nitrogen or its percentage relation. The inanition and gastrointestinal disturbance cannot, therefore, be explained by impaired absorption or by an undue elimination of protein substances. The increased elimination of toxic substances, non-nitrogenous in nature, may be a factor, but upon this point I have no observations.

We now pass to another phase of our subject, the influence of the kidney upon the cardiovascular systems. Here we find that experimental methods have added little to the knowledge based on clinical and anatomical study.

7. *The Effect of Kidney Extracts on Blood Pressure.* Tigerstedt and Bergman, in 1898, detailed a very extensive series of experiments which appeared to demonstrate the presence of a pressor substance in extracts of the kidney of the rabbit. This substance was obtained from the cortex of the kidney and was present not at all or only to a slight extent in the medulla. It could be extracted from the fresh organ by salt solution, by alcohol, by fresh blood, and to a less extent by cold water.

Extracts prepared by boiling gave no effect. The substance was non-dialyzable, and the investigators therefore concluded that it could not be any of the salts of the urine. To this substance they gave the name "renin," and regarded it as an internal secretion of the kidney normally passing into the blood. The rise of pressure, which varied from a few millimeters to 25 to 35 mm. Hg., they believed to be due to an action on the peripheral nerve centres as well as possibly on the spinal cord. Very small amounts caused as much effect as larger doses, and repeated injections produced each an effect as great as the first injection.

In order to demonstrate the passage of this supposed pressor substance into the blood they injected blood from the renal vein into the vessels of rabbits which had suffered double nephrectomy. A moderate rise followed; for example, in one animal twenty-four hours after nephrectomy a rise of 18 mm. Hg. occurred. These last experiments were repeated by Lewandowsky the following year, and although Tigerstedt's rise of pressure was confirmed, Lewandowsky obtained a similar transient pressor effect in his controls, by injecting blood from the general venous (jugular vein) and also arterial systems. The rise he considered to be due in part to the rapidity of injection, but as it was not obtained by injections of salt solution, he concludes that the effect is due to some substance or substances in defibrinated blood capable of pressor effect, but not to an internal secretion of the kidney.

Lewandowsky's results appear to have discouraged the further extensive investigation of Tigerstedt's observations, which one would have expected. The only references in the literature, at least as far as I have been able to determine, are those of Livon, Fiori, Vincent and

Sheen, and of Shaw. Of these, the first two describe a pressor effect; Vincent and Sheen various results, sometimes a fall, sometimes a rise in pressure, and occasionally no effect. Shaw, using the cat, almost uniformly obtained striking and prolonged rise in pressure, varying from 1 to 71 mm. Hg. Mention must also be made of the observation of Oliver, published a year before those of Tigerstedt. Utilizing the exposed frog's mesentery to determine the effect of various organ extracts upon the peripheral vessels, he obtained constantly with adrenal extract a most decisive contraction, but no invariable effect with extracts of the kidney and various other organs.

When one analyzes these investigations, the striking fact is brought out that the result seems to depend on whether or not the kidney extract was injected into an animal of the same species. Thus, Tigerstedt, in a series of about fifty experiments with the rabbit, obtained uniformly a pressor effect, as did also Shaw, using the cat, in all but one of nineteen injections. Vincent and Sheen's injections were not always into animals of the same species, and their results varied. Livon does not give the details of his injections, but his results were uniform, as also appear to have been those of Fiori, whose original paper I have, however, not seen.

The uniformity of these results with kidney extracts, when considered in the light of the fact that other organs, as liver and spleen, appear to contain a depressor substance, led me to repeat these experiments with various modifications in the hope of determining whether or not the pressor effect of the kidney extract was due to a peculiar function of the kidney or to some other factor, physical or chemical in character. The desirability of more light on this question must be evident to any one familiar with the several theories based on the principle of internal secretion, which have been formulated by clinicians to explain the hypertension and heart hypertrophy associated with chronic nephritis. It is sufficient to mention Riva-Rocci, who states that a blood pressure-raising substance is formed in increased amounts in the diseased kidney, and Shaw, who attempts to offer a basis for Traube's theory of uremia by assuming that the substance causing arteriospasm, and thus producing cerebral dis-

turbances without postmortem lesions, is possibly the pressor substance "renin."

The results of my experiments offer no support to the theory that a pressor substance or substances exist in the normal kidney. Early in the investigation a curious contradiction was observed. It was found that the injection into the rabbit of extracts of either dog or rabbit kidney caused a slight rise in pressure, but that similar injection into the dog caused a depressor effect which when dog's kidney was used was very decided. From these observations it was evident that the pressor substance of the kidney of any given species has no constant pressor effect for animals of other species, as is the case with adrenalin. In attempting to analyze these results it was found that almost any substance injected into the rabbit's circulation in doses of 1 to 3 c.c. caused a slight transient rise in pressure. These substances included blood serum, defibrinated blood, urine, extracts of the liver of both rabbit and dog, solutions of urea, sodium chloride, and Locke's solution.

The production of a rise of pressure by such a variety of substances detracts greatly from the significance of the rise following the injection of kidney extracts, and indicates that, in an animal as the rabbit whose circulation is easily disturbed, the effect of the injection is largely if not entirely mechanical. Other experiments demonstrated that the rise in pressure could be obtained with filtrates of the heated extract and with its dialysate, contrary to the statement of Tigerstedt and Bergman's.

The dog's kidney extract was studied in the hope of removing the depressor substance and demonstrating a pressor substance. All such attempts have been unsuccessful. It has, however, been possible to show that the depressor effect is due apparently to the salts of the urine and not to any peculiar constituent of the kidney cells. This was brought out by observations which showed that extracts of other organs of the dog had little or no depressing effect while the urine had a depressor effect equal to or greater than that of the kidney extract. Also the substance was not destroyed by heat or autolysis, was not precipitable by alcohol and dialyzed readily. Experiments to demonstrate the presence of cholin were negative.

8. *Effect on Heart and Blood Pressure of Extirpation of Large Portions of the Kidney.* The recently published investigations of Pässler and Heineke have aroused a new interest in the experimental study of the relation of the kidney to increased blood pressure and cardiac hypertrophy. These investigators found that after the removal of a considerable portion of the kidney substance, approximately two-thirds to three-fourths by successive operations, a rise of blood pressure occurred which was permanent and associated with cardiac hypertrophy. This result was not constant, but occurred in about 25 per cent. of the animals which survived, by at least four weeks, a considerable reduction of kidney substance. In such it was observed also that arterial spasm with further rise of blood pressure quickly followed stimuli which in normal animals would produce little effect. These observations suggest that the heart hypertrophy is due to increased work resulting from the circulatory disturbances caused by a tendency to arterial spasm, and that the vascular spasm is due in its turn to the effect of retained toxic substances. A similar hypertrophy of the heart in dogs after kidney extirpation had previously been observed by Paoli. Bradford states that in dogs with three-quarters of the kidney removed "the blood pressure remains high," even in animals extremely cachectic, but no obvious cardiac hypertrophy was found. There is every reason to believe that the observations of Pässler and Heineke will be confirmed, and if so we will have very valuable evidence of a chemical correlation of extreme importance in explaining the cardiovascular lesions of nephritis. Their confirmation has been a part of my present study, but thus far I have accomplished but little, as most of the animals with considerable kidney reduction have been used for other purposes.

9. *The Effect on Blood Pressure of the Serum of Animals with Experimental Nephritis.* Assuming that if reduction of kidney substance leads to retention of toxic products affecting blood pressure, the acute forms of experimental nephritis would have a similar effect, I have conducted a series of blood-pressure experiments along that line. The serum of dogs poisoned with uranium nitrate and potassium chromate and bichromate has been obtained at various stages of the course of the experimental nephritis and injected in doses of 12

to 20 c.c. into a branch of the femoral veins of normal dogs. The pressure was taken in the carotid. The results have been somewhat surprising. While the serum of a chromate nephritis almost uniformly causes a slight rise in pressure, the injection of the serum of uranium dogs is followed by a decided drop.

That the drop caused by uranium serum is not due to traces of the uranium nitrate injected is shown by the fact that small amounts (0.0075 gr.) have no effect on the blood pressure, while larger amounts (0.0375 gr.) have a pressor effect.

Similar experiments with rabbits give, with both uranium and chromate sera, a definite continuous rise very different from the slight transient rise caused by kidney extracts. In these animals a drop was never observed.

It is evident, therefore, that disturbance of kidney function does cause the appearance of substances in the blood serum which have a definite effect on the blood pressure. The variation in this effect is suggestive. Potassium chromate produces parenchymatous changes in the kidney, while uranium appears to affect both tubular and vascular structures. The difference in action of the two sera may possibly be explained by this difference in disturbance of function. More work along this line is essential.

It matters little whether the substances causing these pressure effects are retained products of metabolism, or are substances increased by the vicarious action of other organs, or, for that matter, are the result of an internal secretion of the kidney itself. It also is a matter of indifference whether the immediate effect is pressor or depressor. The importance is that in disease of the kidney there occurs in the blood serum, in increased amount, a substance or substances affecting blood pressure, and therefore of supreme importance as evidence of chemical correlation in the pathology of diseases of the kidney.

10. *Relation of the Adrenal to Chronic Interstitial Nephritis and Arteriosclerosis.* Of considerable interest at the present moment, in view of the investigations of French clinicians, is the question of the relation which the adrenal bears to the arterial hypertension and degeneration of Bright's disease. It would appear but natural, in view of our knowledge of the influence of adrenalin in raising blood

pressure, of the experimental lesions produced in the rabbit by this substance, and of the clinical phenomena of chronic nephritis, to associate the renal and arterial disturbances with some disturbance of the adrenal. At first glance it is difficult to determine whether the condition in the kidney is responsible for changes in the adrenal leading to an increased outpouring of the secretion and consequent arterial hypertension and degeneration or whether the renal changes are secondary to arterial disease caused by a primary adrenal disturbance. Within the past three years French investigators led by Vaquez have offered a large mass of literature which indicates that nodular adenomata or diffuse hyperplasia of the adrenal is commonly associated with the contracted kidney and arteriosclerosis when the disease does not run too rapid a course. The hyperplasia is considered as an indication of a hyperactivity of the antitoxic and angiotonic functions of the gland, what we might call, perhaps, hyperadrenalism.

To indicate the observations upon which this theory is based a portion of the literature may be reviewed briefly:

The first case described by Vaquez was one of adenoma of the cortex of the adrenal associated with a contracted kidney. Josué described three instances of diffuse arteriosclerosis with hypertrophy of the adrenal. Aubertin and Ambar, in 8 cases of contracted kidney, found in 3 fatty adenomata, and in 4 diffuse hyperplasia; the eighth, with a very rapid course, had a normal adrenal. Lemaire, in a single instance, and Froin and Rivet, in 6 out of 7 nephritics, found adenomata or nodular hyperplasia; the seventh, a patient with but slight rise in blood pressure, was negative. Menetrier found 2 adenomata in 7 cases of contracted kidney. These figures indicate the frequency of changes in the adrenals in association with renal and vascular lesions. There are many negative findings, however, and the frequency of similar lesions with diseases other than those of the kidney and the vascular system have not been sufficiently investigated. Landau who has examined the adrenals in 16 cases of arteriosclerosis finds no changes which might not be ascribed to the effect on the gland of arteriosclerosis itself.

In the hope of throwing more light on the subject by purely anatomical studies, I have examined the autopsy records of the Bender

Laboratory and have attempted to determine the relation, on the one hand, of vascular lesions to changes in the adrenal, and on the other, the association of the latter with chronic interstitial nephritis.¹

The histological examination of a large number of adrenals points conclusively to very definite and fairly constant changes in this organ in general arteriosclerosis. These changes, however, are not limited to the arteriosclerosis associated with contracted kidney but are found, also in the arteriosclerosis accompanying the parenchymatous type of nephritis, and finally in arteriosclerosis without evident chronic lesions of the kidney. The changes are of two types: first, those undoubtedly secondary to the alterations in the vessels of the adrenal, as thickening of the capsule, diffuse increase of connective tissue, and round-cell infiltration; and second, various grades of hyperplasia, nodular and otherwise, variations in the amount of chromaffine substance, and changes in the cytoplasm of the cortical cells.

Whether these latter changes are also secondary to the arteriosclerosis or are independent and perhaps primarily of importance in the production of a hyperadrenalism concerned in the etiology of the vascular lesions I have been unable to determine, although I incline to the former view.

That these various changes occur more or less constantly in association with arteriosclerosis is of great interest and a point of importance in the pathology of this disease and worthy of further study. But in connection with our present study the chief value of these observations lies in the fact that the alterations are not peculiar to chronic interstitial nephritis, with which disease the French particularly associate them, but are found also in other forms of chronic nephritis, and, indeed, in all conditions with advanced arteriosclerosis. It would appear, therefore, upon purely anatomical grounds, that a correlation between the diseased kidney and the adrenal having an influence on the vascular system is doubtful. It must be admitted, however, that this question can only be definitely settled by careful anatomical studies controlled by blood-pressure determinations during life.

¹ For details of this study, see *Journal of Experimental Medicine*, 1908, No. 6.

11. *Adrenalin or Adrenalin-like Substances in the Serum of Nephritics.* Another phase of this subject is the attempt within the past year to demonstrate adrenalin or adrenalin-like bodies in the serum of nephritics. For this purpose has been utilized the observation of the Meltzers that adrenalin causes a dilatation of the frog's pupil. According to Ehremann this reaction is sufficiently precise to allow of the determination of adrenalin in dilutions of 1 to 1,000,000 (0.1 mg. being the minimal blood-pressure-raising dose for the cat).

Schur and Wiesel have found that the serum of patients with chronic nephritis, even when diluted twenty times, causes uniformly mydriasis of the enucleated frog's eye. This result does not occur with the serum of normal individuals or of individuals with other diseases. A similar dilatation is caused by the serum of nephrectomized rabbits, but not by that of the normal rabbit. Eichler confirms the results of Schur and Wiesel in regard to the serum of nephritics and that of nephrectomized rabbits, and considers the question of whether or not the relation is not one of the internal secretion of the kidney to that of the adrenal. Kaufmann, in discussing the results of Schur and Wiesel, states that he obtained a very definite dilatation in two cases of chronic nephritis, but also a slight dilatation with the serum of normal individuals and of individuals suffering from other diseases.

Schlayer has attempted to demonstrate pressor substances by utilizing the "vessel-strip" method. This method, originated by Meyer, consists in fastening a strip of the carotid of the ox, obtained by cutting out a ring of the vessel, to the bottom of a small glass jar, and to the other end a ligature leading to a two-armed lever, one arm of which writes on a smoked drum. Fluids to be tested are placed in the jar containing the "vessel strip." Meyer studied the effect of a variety of substances upon such vessel preparations, and found the vessel to respond by contraction to very minute amounts of adrenalin (0.000015 mg. in 15 c.c. of Ringer's solution). He also found that normal blood serum caused a very definite contraction of the vessel.

Schlayer used this method for testing the serum of nephritics. His control experiments demonstrated the power of normal serum to cause contraction, and his observations on the effect of concentration, dilution, dialysis, and heating had practically the same effect

on the action of the serum as these measures have on the action of adrenalin. He concludes, therefore, that in normal serum is present a substance having some of the physiological as well as the physico-chemical characteristics of adrenalin. Assuming, therefore, that, whether or not this substance is adrenalin, it would be increased in the serum of nephritics if responsible for the increased blood tension, he conducted a series of experiments with sera of patients with various forms of chronic nephritis. Twenty-six observations on sera of eight different patients with blood pressure of 190 to 260 mm. Hg., by v. Recklinghausen's apparatus, were made. Only two sera, one from an individual with a small primary contracted kidney and one from chronic lead poisoning (contracted kidney), gave a reaction greater than that of the control serum. In all others, all contracted kidneys, the effect was less. After ruling out weakening of the active substance by dilution due to the hydremic condition of the nephritic serum, he concludes that the results do not support the adrenalin theory of high pressure in chronic nephritis. Furthermore, he minimizes the importance of Schur and Wiesel's experiment, for, as he argues, as the pupil test is negative with normal serum, while the "vessel-strip" reaction is positive, the substance in nephritic serum causing mydriasis cannot be the same as the blood-pressure-raising substance. In reply to this criticism, Schur and Wiesel suggest that Meyer's method indicates the presence of a pressor substance other than adrenalin, and that, therefore, the subject should be more thoroughly investigated. In support of their own position they emphasize the observation that from the serum of nephritics can be isolated a substance giving the iron chloride reaction for adrenalin.

I have attempted to control these results, but have been unsuccessful, in part, because I could not obtain satisfactory tracings with Meyer's "vessel-strip" method and in part on account of the difficulty of obtaining the sera of nephritics. The frog's pupil test I have applied to the serum of four dogs with severe chromate nephritis, three with uranium nephritis, and one with spontaneous chronic nephritis, with entirely negative results. The serum of uranium and chromate rabbits also gave negative results.

These investigations with the sera of animals with acute experi-

mental nephritis are, however, in no way analogous to those with chronic nephritis in man. The observations of Schur and Wiesel on the one hand and Schlayer on the other are of great importance, and should be repeated by those having access to abundant clinical material.

12. *Nephrotoxic Substances.* The theories concerning nephrotoxic immune serum, or, as it is generally termed, "nephrotoxin," harmonize with some of the statements put forth concerning the internal secretion of the kidney and with the theory of chemical correlation. But a few years ago all kinds of cells were injected in various ways into animals of alien species, in the hope of producing specific cytotoxins for any and all of the tissues of the animal body. The theory underlying these procedures assumed that the injected cells contained a substance capable of stimulating the cells of the injected animal to the formation of antibodies. It assumed virtually, therefore, that the cells introduced contained, for example, in the case of kidney cells, if thoroughly washed free of blood and urine, substances peculiar to cells of that organ and not occurring in the cells of other organs. Such reasoning could well be turned to support the theory of internal secretion, by assuming that the substance causing the immunization was the internal secretion of the kidney set free by the disintegration of the injected cells. The theory of the nephrotoxins, however, was even more comprehensive and the results of such immunization experiments were applied to chronic nephritis. The hypothesis was put forth that in this disease the continued destruction of renal cells led to the formation of a lytic body, autonephrolysin, capable of the continued destruction of other renal cells, a sort of vicious circle, as it were, which explained many of the complications of the disease. Upon this supposition a number of investigators have attempted to demonstrate in animals that the ligation of the vessels of one kidney leads to the escape from the injured kidney of a substance (autonephrolysin) which has the power to impair the function of the other kidney—or that the serum of such an animal introduced into the vein of a normal animal of the same species would cause albuminuria and histological evidence of renal impairment (isonephrotoxin). Although the results of such experiments have been more or less con-

tradictory, they indicate, I think, a theoretical analogy at least between the theory of nephrotoxins and that of internal secretion. The observation of Ascoli, for example, that a nephrotoxic serum caused an increased blood pressure, coupled with the statements of Riva-Rocchi and Maragliano that a similar substance is found in increased quantities in the diseased kidney, has been freely quoted as an explanation of the increased arterial tension and cardiac hypertrophy of patients with renal disease. Unfortunately, however, for this relation, carefully planned experiments do not support the theory of specificity of the cytotoxins. In my own work on this subject, done largely in this city while associated with the University of Pennsylvania, I demonstrated that a true specificity of nephrotoxins did not exist and that the production of autonephrolysin by injuring one kidney was doubtful, and that Ascoli's claim for a blood-pressure-raising substance could not be confirmed. These conclusions have been supported almost uniformly by later investigators.

There is, however, a phenomenon brought out by the investigation of nephrotoxic sera which remains unexplained and which, I think, is of peculiar interest in connection with any discussion of the influence of the normal or diseased kidney on the functions of the body. I refer to the observation of Lindemann that the serum of an animal suffering from an experimental potassium chromate nephritis has the power to produce lesions of the kidney when introduced into a normal animal, and also my own observation of similar results when the serum of dogs with spontaneous nephritis, or of those with lesions due to nephrotoxic immune serum, as has also been noted by Bierry, is introduced into normal dogs. Such injections cause the excretion of albumin and casts with histological changes in the kidney. These observations, which, so far as I am aware, have never been questioned, indicate the presence in the serum of a substance formed anew during a nephritis or accumulating as the result of retention, and therefore of great importance from the point of view of chemical correlation. The phenomenon is quite distinct from that of the action of a substance produced by immunization, as it represents the action of a substance resulting from tissue destruction or faulty function, or both.

Ever since my first experiments on this subject in 1903, I have

intended to take up this problem more in detail, but have had no opportunity until the subject of this address forced it upon me.

Experiments along the general lines suggested I have carried out in association with Dr. H. P. Sawyer. Thus far, in our investigations, we have made nine observations concerning the nephrotoxic action of the serum of animals with nephritis.¹ Two out of three dogs receiving the serum of animals with uranium nephritis and four of five receiving the chromate of potassium serum have given positive results. The serum of a spontaneous nephritis gave a positive result in the one experiment in which it was tried. In all instances the animals were isolated for some time previous to injection and their urine carefully examined for albumin and casts. As I have shown elsewhere this precaution is very important, in view of the frequency with which spontaneous nephritis occurs in the dog. The serum was injected either into a vein or into the peritoneal cavity in doses of 10 to 40 c.c. The elimination of albumin was definite, but usually in small amounts. In but two experiments could it be estimated by the Esbach method, amounting in one experiment to 0.25 per cent. and in the other to 1.5 per cent. Casts appeared sometimes on the first day, but more frequently on the second, and were accompanied by numbers of renal epithelial cells and usually a few white blood corpuscles. This condition of the urine lasted for but a few days, as a rule, although sometimes the return to normal was delayed for a week or more. Control experiments with normal sera were negative.

Similar experiments with rabbits have been tried, but with absolutely negative results. The sera of rabbits with chromate, uranium, and spontaneous nephritis, and of the nephritis produced by injecting nephrotoxic immune serum, have been injected into the ear vein in doses of 5 to 12 c.c. without the occurrence of albuminuria.

The possibility of carrying over in the serum in the experiments with dogs minute amounts of the salts injected must be considered, but it is impossible to detect these salts in the filtrate of the serum concentrated after coagulation. However, the tests employed are not so sensitive as to entirely exclude the persistence of these salts. On the other hand, if present, they would occur in such minute

¹ For completed study, see *Journal of Medical Research*, 1908.

amounts that it seems improbable that they have anything to do with the lesion described. Certainly they appear to have had no effect in the experiments with rabbits.

The direct action of these various sera on renal cells has been determined also by adding the sera to freshly prepared mixtures of kidney cells after the manner carried out in the testing of cytotoxic immune sera. No agglutinative or cytolytic action was evident.

The very definite physiological disturbances seen in these animals in view of the experience of Lindemann and Bierry, give a definite basis, it appears to me, for assuming that the serum of dogs with nephritis contain nephrotoxic substances. In view, however, of the negative experiments with rabbits, it is manifestly impossible to assume that the serum of man also contains these bodies during the course of a nephritis.

13. *Studies of Edema.* Most recent investigations of edema have had to do with the questions of salt retention and water balance—problems to which purely physical methods may be applied. The recent studies of the edema of uranium nephritis, and especially of Heineke's observations upon the apparent power of the serum of an animal poisoned with uranium to produce edema, opens up, on the other hand, the possibility of an explanation of some phases of the problem by chemical correlation.

Uranium nephritis, in rabbits at least, is accompanied, as first shown by Richter, by a well-marked edema of the subcutaneous tissues and hydrops of the pleural and peritoneal cavities; a condition which does not obtain in animals poisoned with chromic salts, cantharidin, aloin, and other renal irritants. But of greater interest from the point of view of chemical correlation is the observation that the serum of an animal with uranium nephritis when introduced into an animal with a chromate nephritis causes the development of a well-marked edema. This phenomenon, first observed by Heineke, and since confirmed by Blanck, who, however, finds it to be not a constant occurrence, suggests that in addition to the presence of nephrotoxic substances in the serum of animals with nephritis there may also occur substances which have an injurious effect on the somatic endothelial cells. It therefore offers a new method of experimenta-

tion for determining the relation of hydremia to renal injury and endothelium destruction in the production of edema. Two explanations seem possible, either the retention, as the result of the kidney insufficiency, of substances which act as lymphogogues of the second order, or the injurious action upon the endothelium of some substance or substances causing an alteration in its permeability to fluids.

In this connection it is impossible to go into the question of the mechanistic *versus* the vitalistic theories of lymph formation. It is sufficient to recall that of the latter theories, Heidenhain's, as well as that of Hamburger, assumes an increased activity of the endothelial cells caused by catabolic products, and that Lazarus-Barlow and Asher believe also in the influence of cell action, but of the cells of the organ rather than of the endothelia. Lazarus-Barlow further emphasizes the influence of waste products. And even Starling, who supports the purely physical theory, assumes an altered permeability of the endothelial membrane.

In connection with these theories of the physiology of lymph secretion we have certain views concerning the pathological secretion of lymph which point to vascular injury as an important factor. Cohnheim and Lichtheim, in their well-known experiments, found that the production of hydremic plethora by injecting large quantities of salt solution into the veins of rabbits and dogs, although it led to edema of the internal organs and ascites, did not cause edema of the normal skin and subcutaneous tissues. But if the skin was irritated, as by exposure to the sun, painting with iodine, or immersion in hot water, local edema of the skin always followed transfusion. From these experiments Cohnheim concludes that the mild irritation of the skin caused an alteration of the capillary walls which made them more permeable for the fluid of the hydremic plethora. Support of this theory is offered by the experiments of Magnus, who found that edema of the skin occurs in transfused animals if arsenic, which pharmacologists consider a specific poison for bloodvessels, is previously injected, or if animals are in deep anesthesia from chloroform or ether. Magnus also found that in nephrectomized animals transfusion, if practised within a day or two, leads to anasarca. Similar results have been obtained by Albu. Closely related to Cohnheim's

theory of renal edema is that of Senator. The difference is that Cohnheim assumes that the altered permeability of the capillary wall is due to the action on these structures of toxic substances not eliminated as the result of the renal insufficiency. Senator assumes that the edema is as much primary as is the renal lesion, and that both are caused by the same toxic agent affecting the glomeruli of the kidney as well as the vessels of the skin, the toxic agent having its origin in the primary disease, as scarlet fever and malaria.

My own experiments have had for their object the production of edema by the administration of substances which would not only produce a renal lesion, but also injure the vessels of the body generally. But more especially the object has been to demonstrate ultimately the presence of endotheliotoxic substances in the serum of animals with experimental nephritis. That is to find support of Cohnheim's theory rather than Senator's, for the former is of greater interest from the point of view of chemical correlation.

Observations of this kind with sera are few in number. Heineke's experience with the serum of uranium animals has been cited. This serum, from animals with edema injected into animals poisoned with chromic salts, which in his experience did not cause edema, produced hydrops of the pleural and peritoneal cavities. His experiments were not reported in detail, but have been confirmed by Blanck, who, however, found that the condition could not be reproduced constantly.

In a later study with Meyerstein, Heineke reports the production of edema in 64 per cent. of the animals receiving uranium serum intravenously, but also found edema in 60 per cent. of those receiving normal rabbit serum. In all instances the animals had been poisoned for four to five days with potassium bichromate, and had received water and sodium chloride by the stomach tube. As this treatment in the absence of serum injection does not cause edema, it would appear that the serum in both instances had some injurious effect on the bloodvessels.

In this connection should be mentioned also the observations of Kast and of Starling upon the lymphagogic action of the serum of edematous nephritics when injected into animals. Kast injected into the vein of a dog 75 c.c. of the serum of a very edematous indi-

vidual suffering from chronic hemorrhagic nephritis, and found the flow of lymph to be increased tenfold. Sera from two other nephritics with edema increased the flow threefold and twofold respectively, while the serum of normal individuals and of nephritics without edema gave no results, as was also the case in one instance each of uremia and cardiac dropsy. Starling reports a single experiment on the dog in which it was observed that the serum of a uremic individual caused a marked quickening of the flow of lymph from the thoracic duct.

In my own experiments, in order to determine if any relation exists between vascular injury and kidney lesion, in the presence of hydremia, rabbits have been treated with substances known to be both renal and vascular poisons, and hydremia has been produced by introducing by a stomach tube considerable amounts (100 c.c.) of water. In other experiments a specific renal poison has been administered first and later a vascular poison. The substances used have been the salts of chromium and uranium, arsenous acid, ricin, snake venom, and nephrotoxic immune serum.

The most important experiments thus far completed have been those with arsenic and nephrotoxic immune sera. Arsenic, a renal and vascular poison, given in the absence of hydremia, produces a local edema about the point of injection which is not observed in simple chromate poisoning, and indicates the toxic action of arsenic on the bloodvessels. When accompanied by daily administration of 100 c.c. of water by the stomach tube there is observed a diffuse gelatinous edema of the subcutaneous tissue of the abdomen with fluid in the thoracic and abdominal cavities. While this result indicates the necessary presence of hydremia in the production of edema, it also indicates the influence of a diffuse vascular poisoning. Diffuse edema after the use of a supposedly specific renal poison as chromic salts I have observed but once. It is, of course, the usual occurrence after the use of uranium, which is supposed to act as a vascular as well as a renal poison.

Peculiarly interesting results have been obtained by the intravenous and intraperitoneal injection of nephrotoxic immune serum into rabbits suffering from chromate nephritis. Control experiments had shown that while normal dog's serum, which is slightly toxic for

the rabbit, did not cause edema in rabbits receiving a large amount of water by the mouth, it did produce in chromate rabbits, receiving the same excess of water, edema about the ureter and the pelvis of the kidney and in the mucosa of the bladder. As the localized edema was due apparently to the well-known toxic action of an alien serum affecting apparently the vessels along the path of elimination, the attempt was made to increase this toxic action by producing a nephrotoxic serum. This was done by injecting rabbits with the washed kidneys of the dog. Such a serum, as is now well known, is not specific in its action; in addition to the nephrotoxic power it has also hemagglutinative and hemolytic properties, and affects, through these activities the vessels of various organs. These latter properties were those most desired. This serum injected into chromate rabbits receiving daily 100 c.c. of water caused uniformly edema of more or less extent. In one instance the subcutaneous edema involved not only abdomen and thorax, but all four legs. The pericardial, pleural, and abdominal cavities contained large amounts of fluid, and the retrosternal and mediastinal tissues and the fat about the pelvis of the kidney were diffusely infiltrated.

To control this observation the same serum was injected into normal rabbits receiving an excess of water, with no result except a moderate but very definite edema of the retrosternal tissues, with in one instance a small amount of fluid in the pleural cavities.

A shorter series of experiments along the same general lines was made on dogs except that water was not administered in excess. The animals were well supplied with water, but none was administered by the stomach tube. In none was it possible to produce edema.

These experiments are as yet incomplete, and I am not prepared to draw definite conclusions. They confirm, however, the general opinion concerning the relation of hydremia to the kidney lesion in the production of edema, and to my mind point very strongly to the important part played by vascular poisons. The application of data derived from experiment to human pathology is not always safe, but these observations would appear to support the supposition that toxic substances accumulating in the blood may aid in the production of edema by an injurious action on vessel endothelium.

In conclusion, it is evident, I think, from this critical review and from the experiments which have been presented, that there is little to support the older theory of the internal secretion of the kidney, but, on the other hand, much to indicate that the application of the theory of chemical correlation to the pathology of chronic nephritis may aid in elucidating many doubtful phases of this disease. The evidence of such correlation, although not conclusive, is sufficient to warrant the utilization of all experimental methods, but especially those of physiology and biological chemistry, in the hope of eventually adding to our knowledge of the obscure principles concerned in the production of the important lesions associated with chronic diseases of the kidney. The presentation, in an orderly manner, of the possibilities of investigation in this territory and of some of the methods of attack have been the principal objects of this address.

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The Interpretation of the Venous Pulse.¹

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THE knowledge of the existence of pulsations in the veins dates back to 1704, when Homberg² presented to the French Academy the result of his observations on the venous pulse. Morgagni,³ however, was the first to offer rational views concerning this phenomenon.

¹ Read by invitation.

² Sur un battement de veines semblable au battement des artères. *Hist. acad. roy. d. sc.*, Paris, 1704. *Amst.* 1707, 218 to 223. Also, *Rec. d. mem.*, Dijon, 1754, ii, 138 to 140.

³ De sedibus et causis morborum, Venice, 1762. Letter XVIII, Sec. 9, 10, 11.

His dissertation upon the venous pulse is to be found in his work, *De sedibus et causis morborum*, which appeared in Venice in 1762. The first attempts to study the venous pulse experimentally were made by Barry¹ in 1826 and by Wedemeyer² in 1828, but owing to the inadequate methods used, their results were rather unsatisfactory. Bamberger³ in 1856, and others following him, in common with their clinical predecessors, described the venous pulse as occurring only in tricuspid insufficiency. Bamberger later (1863) published tracings of the venous pulse of tricuspid regurgitation, while Marey⁴ in the same year took graphic records from animals. Friedreich,⁵ in 1865, showed its presence in other conditions than tricuspid insufficiency, and described it as being formed of two waves: one presystolic in time and due to the contraction of the auricle, the other systolic in time and due to the shock of the aorta against the superior vena cava. Potain,⁶ in 1868, described the venous pulse as composed essentially of a positive wave, due to the auricular systole, followed by two negative waves, the first being caused by the auricular, the second by the ventricular diastole. The same observer, in 1881, published tracings taken simultaneously from the jugular vein, and either the carotid artery or the cardiac apex. In the same year Riegel⁷ interpreted the normal venous pulse very much as Friedreich had before him, believing, however, that the principal wave was that originating in the contraction of the auricle.

Since that time most of the descriptions given in text-books on diagnosis, have embraced two forms of venous pulse: (1) The physio-

¹ Recherches expérimentales sur les causes du mouvement du sang dans les veines. Mem. Acad. d. Sc., Paris, June 8, 1825. Paris, chez Crevot, 1825. Experimental researches on the Influence of Atmospheric Pressure upon the Progression of the Blood in the Veins, etc., London, T. and G. Underwood, 1826.

² Untersuchungen über den Kreislauf des Blutes und insbesondere über die Bewegung desselben in den Arterien und Capillargefässen, Hanover, 1828.

³ Lehrbuch der Krankheiten des Herzens, 1857. Beobachtungen über den Venepuls. Würzb. med. Ztschr., 1863, iv, 232.

⁴ Physiologie méd. de la circulation du sang, 1863.

⁵ Ueber den Venepuls. Deut. Arch. f. klin. med., 1865 to 1866, i, 241.

⁶ Des mouvements et des bruits qui se passent dans les veines jugulaires. Mem. Soc. med. Hop. de Paris, 1867 and 1868.

⁷ Ueber den normalen u. pathologischen Venepuls. Deut. Arch. f. klin. med., 1882, xxxi, 1. Experiment. Untersuchungen über den normalen Venepuls. Deut. Arch. f. klin. med. xxxi, 470.

logical venous pulse, characterized by a presystolic positive wave attributed to the arrest or slowing of the flow of the blood in the veins as a result of the auricular contraction; since this wave is immediately followed by a negative wave synchronous with the arterial pulse, this form of venous pulse is sometimes called systolic venous collapse; (2) the pathological or centrifugal regurgitant venous pulse consisting of a systolic positive wave due to a regurgitation of the blood into the auricle, and, possibly, also into the veins during ventricular systole and characteristic of tricuspid insufficiency, whether it be organic or functional.

François Franck¹ and later Fredericq² showed that in the absence of tricuspid regurgitation a slight systolic wave is constantly present in the jugular veins of animals. Franck attributed the formation of this wave to the shock of closure of the auriculoventricular valves, while Fredericq believed it to be due to the sudden projection of these valves into the auricle at the beginning of ventricular systole. They both interpreted the presystolic wave as their predecessors had: as caused by the auricular systole. They also called attention to a third wave occurring at the end of ventricular systole. François Franck ascribed it to the sudden lowering of the base of the heart at this phase of its cycle. Fredericq assigned its cause to the sudden diminution in volume of the auricle occurring when its walls return to their position of rest.

To Mackenzie³ belongs the credit of having attracted the attention of clinicians to this third wave, which had escaped their notice until his excellent work, *The Study of the Pulse*, appeared.

The apparatus used to obtain the tracings shown in this article consists of two Marey tambours provided with long levers made of very thin, light straw, the writing points being short and thin strips of aluminum. The pulsations were taken up by means of Mackenzie's

¹ Mouvements des veines du cou en rapport, etc. Gaz. hebd. med. et chir., Mars et Avril, 1882. Nouvelles recherches sur un cas d'ectopie cardiaque pour servir à l'étude du pouls jugulaire. Arch. de phys., 1889, 1, p. 70.

² Sur le pouls veineux physiologique, Travaux du laboratoire, 1889 to 1890, t. iii, p. 85. La pulsation du coeur chez le chien. Arch. de biologie, 1890, x.

³ The Significance of the Venous Pulse. Edinb. med. Jour., 1894. The Study of the Pulse and Movements of the Heart, London, MacMillan, 1903. The Interpretation of the Pulsations in the Jugular Veins. Amer. Jour. Med. Sci., 1907, cxxxiv, p. 12.

capsule, or by means of a small glass funnel, according as the one or the other gave the best results. So far as the venous pulsation is concerned, Mackenzie's capsule with a flat side which can be laid against the clavicle, is best adapted for the purpose. Needless to say, the rubber tubes leading from the receivers (capsules or glass funnels) to the tambours were exactly of the same length. Especial care was also taken before each observation to see that the writing points fell exactly on the same perpendicular line. The kymograph, on which the records were made, is the latest model of the Harvard kymograph, the speed of which can readily be varied to suit each particular case.

The time record was obtained by means of a Page vibrator, the reed of which vibrated fifty times a second.

The superiority of such an arrangement is obvious when one compares the results here presented with the tracings taken with even the best of the small sphygmographs. The clockwork of the latter instruments is often too slow to permit of an accurate estimation of slight differences of synchronism between two curves, a point which is not without importance, as we shall see later.

The venous pulsation was taken from the point at the root of the neck which gave the best excursion of the lever. In the great majority of instances this point is to be found on the right side, although in a certain number of cases the venous pulse is more marked on the left side. The best pulsating point is often over the position of the jugular bulb, but it is by no means rare that a better jugular pulse can be recorded when the receiver is placed over the lower part of the external jugular vein. Finally the patient was placed in the recumbent posture, for in many cases the jugular pulse disappears when the patient assumes a posture in which the trunk is erect. As dyspnea is a disturbing factor, whenever this was present the patient was asked to breathe as quietly as possible.

For the purpose of timing the various events in the complete cycle of a jugular pulsation, a simultaneous record of an arterial pulse or of the heart beat is indispensable. The carotid pulse was, therefore, taken simultaneously with the jugular pulse, and in all those cases in which it was possible a record of the heart beat was also taken simultaneously with the jugular pulsation. In order to establish points of synchronism in the two curves, ordinates were drawn with the levers

while they were still in the position they occupied during the time the tracing was being taken.

In Fig. 1 the complete cycle of a jugular pulsation is seen to consist of three positive waves, the first two of which, *a* and *s*, are relatively of short duration and have a sharp summit, while the third wave, *v*, is of longer duration and has a broad summit divided by a notch so that it presents a bifid appearance. These positive waves are separated

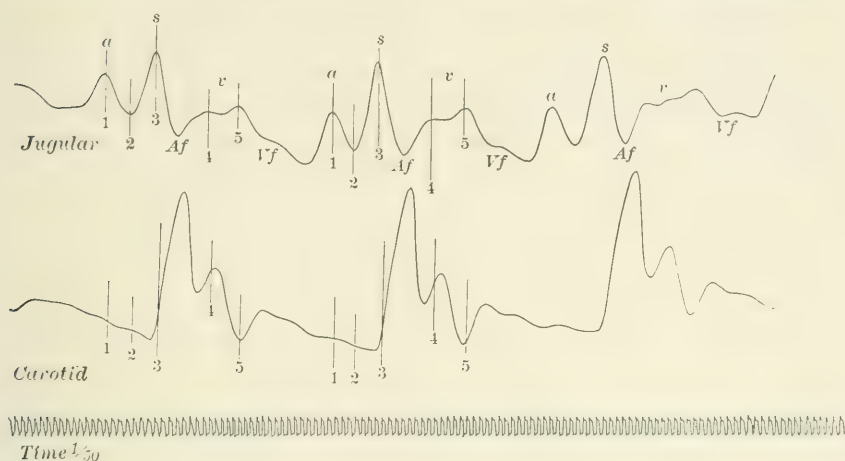


FIG. 1.—Simultaneous tracings of the jugular and the carotid pulses. From a case of mitral insufficiency and stenosis with perfect compensation. (Was admitted to the hospital for hydrops of the gall-bladder.) *a*, auricular wave; *s*, systolic wave; *v*, ventricular wave; *Af*, auricular filling; *Vf*, ventricular filling. The ordinates having the same numbers mark synchronous points on the curves. The letters and numbers have the same significance in all the tracings.

by three depressions or negative waves. The first two (*a* and *Af*) are abrupt and occupy little time, while the third (*Vf*) situated between wave *v* and the next jugular pulsation, is more gradual in its descent, is deeper, and occupies a longer time in the cycle.

The first positive wave, *a*, is presystolic in time and is invariably interpreted as being due to the contraction of the auricle. The mechanism of its causation, however, admits of several interpretations. It may be due to the slowing or arrest of the venous flow with a resulting damming of the blood and increase in the diameter of the veins, or to the suddenness with which the blood stream is stopped, this

giving rise to a wave travelling in a reverse direction. It may also be due to the propagation of a wave of regurgitation from the auricle.

Certain investigators, as Fredericq and Nuel,¹ do not believe that any regurgitation occurs into the great veins during auricular systole, under physiological conditions. On the other hand, Chauveau and Faivre² state that in the horse there is normally a slight regurgitation of blood into the great veins during the contraction of the auricle. Keith³ advances the opinion that the mouth of the superior vena cava is normally closed during the systole of the auricle by the contraction of a muscle band, the *tinea terminalis*, which performs the function of the venous valves found in the hearts of reptiles and batrachians. Should the auricle become engorged, however, the relaxation of this

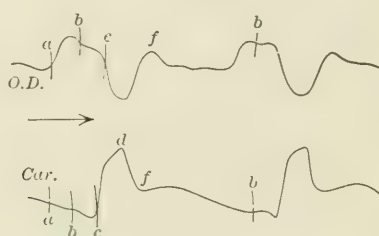


FIG. 2.—Simultaneous tracings of the intra-auricular pressure (*O.D.*) and of the pressure in the carotid artery (*Car.*) of a dog (morphine anesthesia). (Fredericq.) *a b*, auricular systole; *b c*, beginning of ventricular systole and projection of the auriculo-ventricular valves toward the auricle; *c d f*, outflow time; *f*, closure of semilunar valves (dicrotic notch).

muscle band would allow of regurgitation into the veins, and MacKenzie thinks that it is easily rendered incompetent by changes affecting the tonicity of the auricle.

It is probable, however, that in the normal state the main factor in the formation of this wave is to be found in the slowing of the venous current, and the resulting accumulation of the blood in the

¹ *Elements de physiologie humaine*, Gand, 1893.

² *Nouvelles recherches experimentales sur les mouvements et les bruits normaux du coeur, envisagés au point de vue de la physiologie médicale.* Gaz. med. de Paris, 1856, iii série, t. XI, p. 406.

³ *The Evolution and Action of Certain Muscular Structures of the Heart.* Lancet, February 27, 1904, and March 5, 1904.

veins, and, possibly, also in the production of a centrifugal wave originating through the sudden arrest of the inflowing blood.

The negative wave seen between *a* and *s* is easy of explanation. It is due to the collapse of the veins occurring with the resumption of the flow of the blood consequent on the relaxation of the auricle. This fall in the curve is, however, quickly interrupted by the positive wave, *s*, the interpretation of which has given rise to a great deal of controversy. It will be noticed that it is systolic in time, more correctly protosystolic, for it is normally synchronous with the beginning of ventricular systole. It reaches its height in the interval comprised between the beginning of the contraction of the ventricle and the opening of the semilunar valves, in other words, in that part of the cardiac cycle designated by the French as "systole préparatoire," and by the Germans as "verschlusszeit" (Figs. 1, 2, and 4).

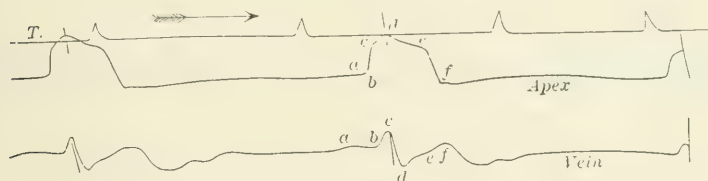


FIG. 3.—Simultaneous tracings of the apex beat and of the jugular pulse obtained from a large dog (morphine anesthesia). (Fredericq.) *a b*, auricular systole; *b c*, beginning of ventricular systole and projection of the auriculoventricular valves toward the auricle; *c d e* (on apex tracing), systolic plateau; *e*, closure of semilunar valves; *e f*, relaxation of ventricles; *T*, time in seconds.

This wave was formerly considered abnormal and as being due to tricuspid regurgitation. This has been shown to be incorrect, as the jugular veins of normal animals exhibit it. Several explanations have been offered concerning its mode of production. Friedreich attributed it to the impact of the aorta against the superior vena cava. As already stated, François Franck believed it to originate in the sudden closure of the tricuspid valve, while Fredericq thought it was due to the projection of these valves toward the cavity of the auricle. Gerhardt¹

¹ Klin. Untersuchungen über Venenpulsationen. Arch. f. exp. path. u. pharm., xxxiv, 402. Einige Beobachtungen am Venenpuls, Ibid., 1902, xlvii, 250.

does not think that this latter explanation is warranted. He considers that the auriculoventricular opening is so tightly closed by the sphincter action of the ring of muscle around it, that as a result the valve leaflets are kept almost completely in apposition and that, therefore,

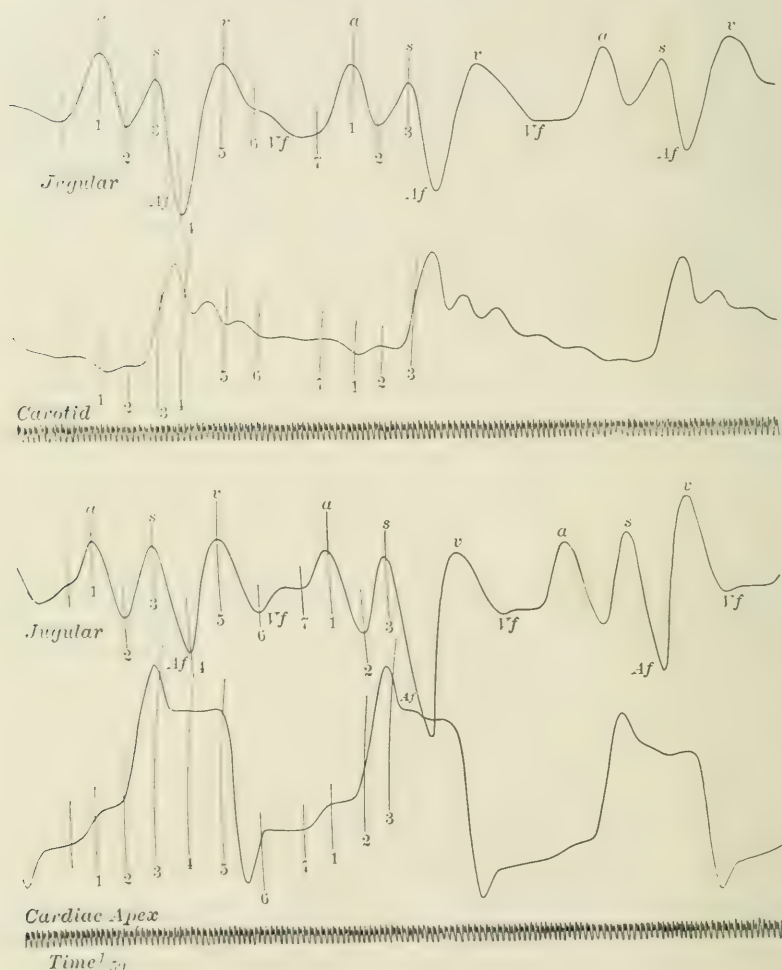


FIG. 4.—Simultaneous tracings of the jugular and the carotid pulses and of the jugular pulse and apex beat. Note that the upstroke of wave *s* coincides with the upstroke of the ventricular contraction (ordinate 2). Accordingly wave *s* precedes the carotid pulse by about $\frac{1}{10}$ second. Wave *v* in this case is single. (Case of aortic regurgitation and relative mitral insufficiency with failing compensation.)

they could not be pushed toward the auricular cavity by the sudden rise in ventricular pressure due to the contraction of the ventricle. He accordingly prefers Friedreich's explanation that the arteries coursing alongside the veins animate them with their own pulsation. Mackenzie adopted a similar interpretation, and he attributes the production of this wave as seen in the jugular pulse to the communicated impact of the neighboring carotid artery; hence the name he gave to it of "carotid wave." Mackenzie has advanced a number of arguments in support of his view. It is not within the scope of this paper to take them and discuss them seriatim. Those interested in the subject will find the necessary information in papers by Morrow¹ and by Bard.²

No one will dispute the fact, however, that the pulsation of a contiguous artery may contribute to the formation of this wave. However, I do not believe that it arises entirely from this source, but rather that it is independent of it, and that all the pulsation of a neighboring artery can do is to add itself to it, or vitiate it. Bard states that he obtained this wave from a vein in front of the clavicle and that it occurs in those cases where the carotid is pulseless, provided the ventricles do not fail to contract. Animal experimentation has made this point clear. Fredericq, Morrow, Cushny and Grosh³ and others have shown that this wave can be obtained when methods of recording the jugular pulse are used that do not involve pressure over the carotid artery, as well as when the vein is carefully dissected away from the artery, or when the carotid is clamped close to the aorta. The objection can be raised, however, that these methods do not eliminate the influence of the aorta on the vena cava. An examination of the curves of intra-auricular pressure obtained by Marey, Fredericq (Fig. 2), Porter⁴ and others shows the presence of this wave in the position mentioned above. Porter ascertained that when the ventricle is

¹ The Venous Pulse. Brit. Med. Jour., 1907, 777. The Various Forms of the Negative or Physiological Venous Pulse, *ibid.*, 1906, 1807.

² De l'enregistrement graphique du poulx veineux des jugulaires chez l'homme. Jour phys. et path. gener., 1906, t. VIII. Des divers détails du poulx veineux des jugulaires chez l'homme, *ibid.*

³ The Venous Pulse. Jour. Amer. Med. Assoc., No. 15, xlix, 1254.

⁴ Researches on the Filling of the Heart. Jour. Physiol., Cambridge, 1892, xiii, 513 to 553.

inhibited by stimulation of the vagus, this wave does not appear in the curve; he therefore concluded that it is due to the pushing of the

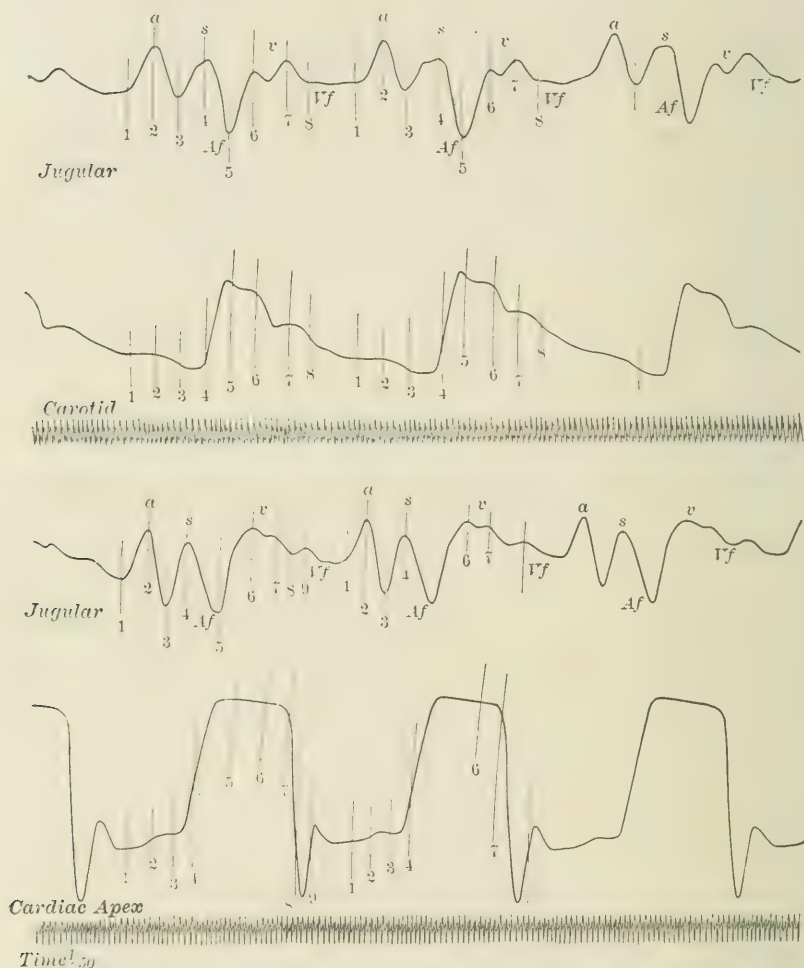


FIG. 5.—Simultaneous tracings of the jugular and the carotid pulses and of the jugular pulse and apex beat. From the same patient as Fig. 4 during a period of improvement.

auriculoventricular diaphragm toward the auricular cavity. The venous tracings illustrating this article show a striking resemblance to

the curves of intra-auricular pressure given by Porter in his valuable paper.

That wave *s* is really independent of any arterial pulsation is shown by the fact that in the majority of cases it precedes the systolic line of ascent of the carotid pulse. So far as I am aware, Bard was the first to direct our attention to this point. An examination of the tracings shown here will reveal the truth of this assertion. In order to demonstrate this the tracings must be taken on a kymograph revolving at a sufficiently rapid rate to bring into evidence little differences of synchronism in the curves, and synchronous points should be marked with the levers in the manner already described, or by any other accurate method. The time elapsing between the beginning of wave *s* and the beginning of the carotid pulse varies in accordance with the degree of completion of the line of descent of the auricular wave, the rapidity with which the ventricle responds to the stimulus coming from the auricle as well as the rapidity and ease with which the ventricle overcomes the pulmonic pressure. This is well exemplified in Fig. 6, taken from a case of mitral stenosis in a fair state of compensation. According to Bard the difference in time between the appearance of this wave and the carotid pulsation varies from $\frac{1}{100}$ to $\frac{2}{100}$ of a second. Until the present time most of the tracings I have been able to obtain show somewhat higher figures, $\frac{4}{100}$ to $\frac{6}{100}$ of a second; in a few instances, however, Bard's figures were confirmed. In the middle group of Fig. 6, the time interval between ordinate 2 and the beginning of the carotid pulse is $\frac{2}{100}$ of a second.

Following the protosystolic rise *s*, a sudden fall occurs in the tracing producing the negative wave *Af*. This wave is generally attributed to the rapid emptying of the veins which takes place during the diastole of the auricle. The negative pressure in the auricle is due, not only to the dilatation of its walls, but in great part also to the action of the contracting ventricle. Chauveau and Marey¹ observed that the auriculoventricular septum is displaced toward the apex during the systole of the ventricle, this having for effect an enlargement of the auricular cavity. Porter offered another explanation of the production of a negative pressure in the auricle based upon the investigations of

¹ Appareils et expériences cardiographiques. Mem. acad. med., Paris, 1863, t. xxvi 313.

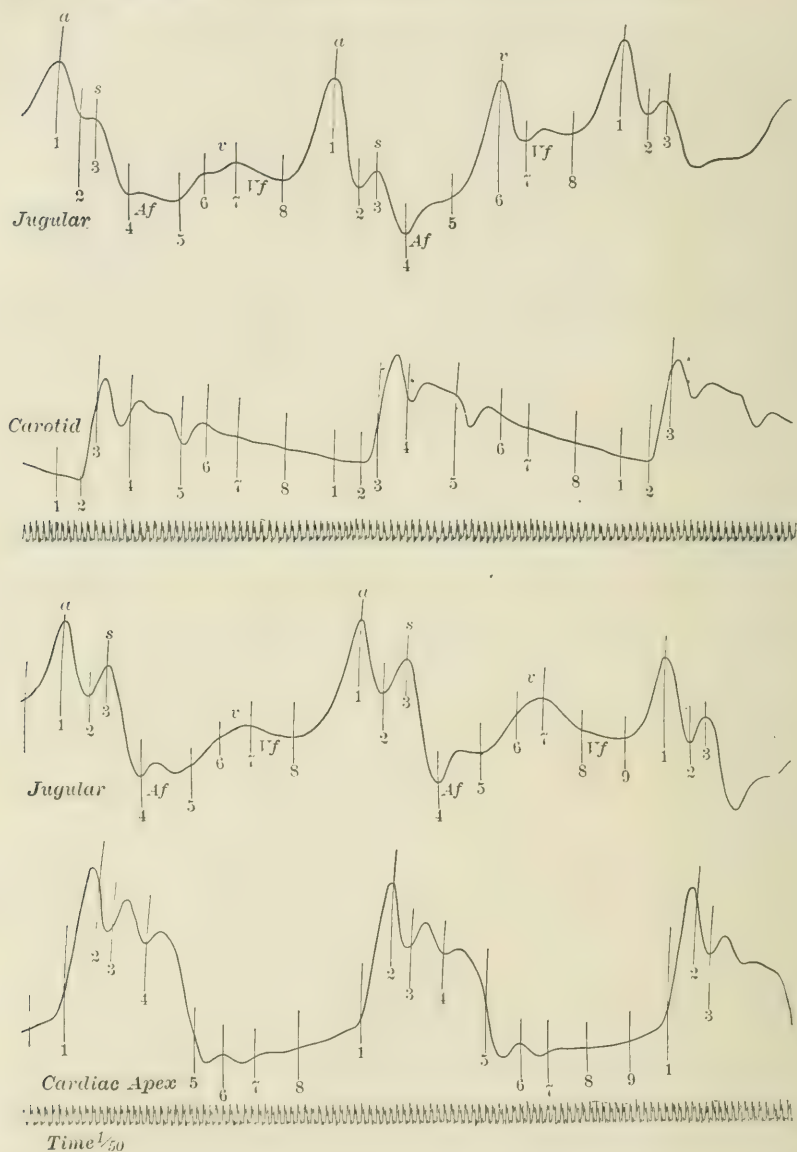


FIG. 6.—Simultaneous tracings of the jugular and the carotid pulses and of the jugular pulse and apex beat. Wave *v* occurs later in the cycle than in the other tracings. With the exception of the middle group, wave *s* begins with the carotid pulse. An examination of the lower tracings show that the ventricle responds quickly to the auricular impulse; wave *s* therefore occurs on the descending limb of wave *a*. (Case of mitral stenosis and regurgitation with beginning failure of compensation.)

Purkinje, Nega, and Roy and Adami. He believes that it is due to the contraction of the papillary muscles pulling the auriculoventricular valves toward the ventricle.

Some investigators have thought that this negative wave was caused by changes in intrathoracic pressure coincident with the change in the volume of the heart occurring during ventricular systole. Gottwalt¹ showed this conception to be incorrect, inasmuch as this negative wave persists even though the chest be opened.

This wave then is indicative of the filling of the auricle, and is the product of several factors: the expansion of the auricle, the downward movement of the auriculoventricular septum, and the pull exerted by the papillary muscles on the auriculoventricular valve leaflets. It begins with the opening of the semilunar valves and lasts until toward the end of ventricular systole, when the third positive wave makes its appearance.

The normal position of the third positive wave, usually called ventricular wave *v*, is toward the end of ventricular systole. However, different observers do not agree to this, and some, like Gerhardt, believe it to occur during ventricular diastole. The fact is that the relation of this wave to the cardiac cycle is not a perfectly constant one, and that pathological conditions within the heart seem to influence both its position and its form. It is not the purpose of this paper to discuss the pathological modifications of the venous pulse; we will, therefore, consider it only as it occurs normally in the jugular veins of animals, and in those cases in human beings who exhibit venous pulsations either without apparent pathological cause or as a result of some slight venous embarrassment.

Various explanations have been offered concerning the interpretation of this wave. Some, like Riegel, think that it is due to the communicated shock of the arterial dicrotic expansion; others, among whom is François Franck, see in it the effect of changes in intrathoracic pressure owing to the diastolic enlargement of the ventricle. But it has been shown that opening the thorax in animals does not affect the production of this wave. Mackenzie and others believe that it is due to the filling of the auricle and the consequent accumulation of the

¹ Der normale Venenpuls. Arch. f. die ges. physiol., 1881, xxv, p. 1 to 30.

blood in the veins. Mackenzie, however, associates it in some way or other with tricuspid regurgitation.

While it is in all probability true that tricuspid regurgitation affects the *v* wave, it is none the less true also that it occurs in cases where there is no regurgitation. It is found as a constant wave in the jugular pulse of animals, as well as in the curve of intra-auricular pressure. Moreover, the mechanism of its production is to be sought in the movements of the ventricle. Fredericq, Gerhardt, Wenckebach, and others have ascribed it to the swinging back of the auriculoventricular septum. Morrow does not believe that the action of the ventricle is necessary for the formation of this wave, but Cushny and Grosh have shown that the suspension of the ventricular contractions is attended with the disappearance of this wave from the tracing.

It was stated above that two of the factors contributing to the formation of the negative wave *Af* were, on the one hand, the displacement of the auriculoventricular septum toward the apex, and, on the other hand, the pull of the papillary muscles on the tricuspid leaflets.

The relaxation of these papillary muscles and the return of the auriculoventricular septum to its former position decrease the cavity of the auricle, and, as the latter is filled, this encroachment upon its capacity has for effect an arrest of the flow and a damming of the blood in the veins. These two movements are, however, not synchronous, and, according to the researches of Roy and Adami,¹ the papillary muscles relax shortly before the beginning of ventricular diastole, hence before the return of the auriculoventricular septum to its position of rest. This relaxation occurs while the intra-ventricular pressure is still much higher than the intra-auricular pressure. We would therefore expect these two separate movements to be reflected in the wave under consideration. That this may be as here explained is seen by the constancy with which a notch is present on the summit of wave *v*, thus showing it to be made up of two lesser waves.

The variations in the form and in the position of this wave in the cardiac cycle are to be sought in pathological factors which may mask or annul the effect of the movements just described.

¹ Heart Beat and Pulse Wave. Practitioner, London, 1890, xlv, 81, 161, 241, 347, 412; xlv, 20.

The third negative wave in the jugular pulse *Vf* occurs at the beginning of ventricular diastole, and is due to the opening of the tricuspid valve; the auricular blood entering the relaxing ventricle, the flow of the blood in the veins is accelerated, and the latter naturally decrease in diameter.

In almost all tracings taken from normal animals, either from the jugular vein or from within the auricle, this wave is more shallow than the negative wave *Af* (auricular filling). Although the tracing of Fig. 5 was taken from a pathological case of aortic and mitral regurgitation, what may be taken as the normal ratio of the depth of these two waves is well shown there.

It seems at first strange that this negative wave should be so little marked, but if it be remembered that the base of the ventricle may still be continuing its upward movement, though perhaps more slowly, it will be easily understood how this would counteract the effect of the aspirating action of the ventricle by gradually decreasing the capacity of the auricle.

A slight positive wave is very constantly present at or near the trough of this negative wave. It is in all probability a wave of stasis due to the entrance of blood into the veins from the periphery.

A moment's consideration will make it apparent that the study of the venous pulse by the graphic method is destined to give valuable information concerning the pathological function of the heart, for it can reasonably be inferred from what has been said, that it mirrors, more faithfully than the arterial pulse, the events occurring within the heart. Already it has proved invaluable in the study of heart-block, and in the study of the various forms of arrhythmia.

CONCLUSIONS. The physiological or so-called negative systolic venous pulse consists of three positive and three negative waves bearing a more or less definite relation to the events of the cardiac cycle and having their origin in the various movements of the chambers and structures of the right heart. The first positive wave (*a*) is pre-systolic in time and is due to the contraction of the auricle causing a slowing of the venous current and producing a centrifugal wave through a sudden arrest of the inflowing blood. The second positive wave (*s*) is protosystolic in time and originates in the sudden projection of the tricuspid valve into the cavity of the auricle during the quick,

incipient rise in intraventricular pressure occurring in the protosystolic period. The third positive wave (*v*) occurs toward the end of ventricular systole. It consists of two lesser waves separated by a shallow notch. The factors entering into its formation are the relaxation of the papillary muscles at a time when the intraventricular is still higher than the intra-auricular pressure, this resulting in an upward movement of the tricuspid leaflets, and to the return of the auriculoventricular septum to its position of rest.

The first negative wave (between positive waves *a* and *s*) is due to the relaxing auricle. The second negative wave (*Af*) occurs during the diastole of the auricle. It is due to the dilatation of its walls, to the displacement of the auriculoventricular septum toward the apex occurring at the time of ventricular systole, and to the pull of the papillary muscles on the tricuspid valve leaflets. The third negative wave (*Vf*) appears during ventricular diastole and in the common pause of the heart chambers. Its cause is found in the passage of the blood from the auricle into the ventricle. It is somewhat modified, possibly by the continued ascent of the auriculoventricular septum and by a wave of stasis due to the accumulation of blood coming from the periphery.

I wish here to thank Professors A. P. Brubaker and W. M. L. Coplin for their encouragement and advice, and Professors J. C. Wilson and H. A. Hare from whose wards the necessary material was obtained.

April 9, 1908.

A Study of the Proteolytic Ferments of the Large Lymphocytes in a Case of Acute Leukemia.

BY WARFIELD T. LONGCOPE, M.D., AND J. L. DONHAUSER, M.D.

(From the Ayer Clinical Laboratory of the Pennsylvania Hospital.)

AT least thirty years ago chemical investigations made upon the blood of persons suffering from leukemia seemed to show that in those forms of the disease characterized by the presence of granular myelocytes peptone was formed in the blood. Schumm,¹ in 1903, pointed out that peptones were only formed in the blood after it had been shed, while Erben,² at the same time, demonstrated that the leukocytes from the blood of cases of myelogenous leukemia contained a ferment which was capable of digesting fibrin. Digestion took place best if the fibrin and cells were suspended in an alkaline fluid, though some evidence of the proteolysis could be seen when acid was used. That both the polymorphonuclear leukocytes and the neutrophilic granular myelocytes contain enzymes that are capable of digesting blood serum at 55° or 60° C. or gelatin at 36° C. has been amply demonstrated by Jochmann and Müller³ and Stern and Eppenstein.⁴ These investigators have found repeatedly this enzymotic action in the cells from the blood of cases of myelogenous leukemia, but have failed to demonstrate that the lymphocytes either from cases of lymphatic leukemia or the lymph nodes from cases of pseudoleukemia possess any such action.

Eppenstein⁵ was unable to show any biological differences between the large lymphocytes of the blood from a case of acute lymphatic leukemia and the small lymphocytes from cases of chronic lymphatic leukemia. Luksch⁶ found in two cases of myelogenous leukemia, both of which had very high leukocyte counts, that a drop of blood produced cupping of the surface of coagulated blood

¹ *Beit. zur chem. Physiol. u. Path.*, 1903, iv, 442.

² *Zeit. f. Heilk.*, 1903, xxiv, 70; *Beit. zur chem. Physiol. u. Path.*, 1904, v, 461.

³ *Münch. med. Woch.*, 1906, liii, 1393, 1507, 2002, 2093.

⁴ *Ibid.*, 2192.

⁵ *Deutsch. med. Woch.*, 1907, xxxiii, 1984.

⁶ *Folia Haemat.*, 1908, v, 75.

serum at 55° C. Blood from a case of what he terms lymphatic leukemia, showing a leukocyte count of 8600, 51 per cent. of which were mononuclears and 49 per cent. polynuclears, gave no digestion. The diagnosis in a second case was somewhat doubtful, for there was no study of the blood, but the lymph nodes were enlarged and contained large lymphocytes and there were nodules in the spleen. The cells of the bone marrow and the spleen contained proteolytic ferments, but the lymph glands were without enzymotic action. In a third case the leukocyte count was 180,000. Of the white cells 98 per cent. were leukocytes, half of them small lymphocytes and the other half large, while all degrees of transition between the two were observed. The blood from this case did not digest blood serum. Luksch's conclusions, that the large lymphocytes in cases of lymphatic leukemia are without ferments and act, therefore, like the small lymphocytes, do not seem to be entirely justified by his experiments, for in the first case there were probably not sufficient white cells in the drop of blood, otherwise one would expect to see some evidence of act on from the polymorphonuclear leukocytes, which are known to contain ferments and which were present in approximately the same numbers as the lymphocytes. The diagnosis in the second case is too uncertain to enable one to draw conclusions, while the third case was evidently not one of the pure large cell type of leukemia. Müller¹ states that in one case of acute lymphatic leukemia the cells acted upon blood serum coagulated at 55° to 60° C. in the same manner as polymorphonuclear leukocytes, digesting the proteid when the reaction was alkaline or neutral.

Opie² has demonstrated the importance of the reaction of the fluid in which the leukocytes are allowed to act. He has observed that the cells obtained from pleurisies produced experimentally in dogs show two types of digestion, corresponding to the two varieties of cells found in the exudates. The enzymes derived from the polymorphonuclear leukocytes or microphages act best in an alkaline or neutral solution, whereas the other enzymes derived from the large phagocytic cells or macrophages act almost exclusively in an acid reaction. In neutral or alkaline solutions Opie found that emulsions

¹ Deutsch. Arch. f. klin. Med., 1907, xci, 291.

² Jour. of Exper. Med., 1905, vii, 316; 1906, viii, 410; 1907, ix, 391 and 414.

of lymph glands from dogs, rich in these large phagocytic cells, have practically no proteolytic action, while in dilute acid suspension the emulsions showed definite enzymotic activity.

In the presence of fresh unheated blood serum the leukocytes and large phagocytes are practically inactive, for the blood serum, as has been demonstrated by Opie,¹ Jochmann and Müller,² Wiens,³ Bittorf,⁴ and Wiens and Müller,⁵ contains an antiferment which though injured when heated to 55° to 60° C. is not entirely destroyed until the serum is heated to 75° C. for several minutes.

In view of the discussion as to the origin of the large lymphocytes in acute lymphatic leukemia and their relationship to the granular myelocytes on the one hand and the non-granular lymphocytes on the other, it seemed of importance to establish, if possible, the position of these cells on a biological basis.

Since it was necessary to study the leukocytes of the circulating blood, a method had to be devised for obtaining the white cells as free as possible from the admixture of the red corpuscles. It was, moreover, important to free the white cells from the serum, on account of the property which the blood serum has of inhibiting the enzymotic action of the leukocytes. To procure the lymphocytes in as great a concentration as possible, the blood, drawn from the arm vein, was diluted with several times its volume of 1.5 per cent. sodium citrate, the mixture centrifugalized, and the upper buffy layer withdrawn. As could be determined by microscopic examination, this layer contained all the leukocytes. The mixture of leukocytes and red blood cells was then washed three or four times in 0.85 per cent. sodium chloride suspended in a given quantity of this solution and used for the experiments. In a few instances 1 per cent. ammonium oxalate was employed instead of sodium citrate. As the ammonium oxalate destroyed the red blood cells, the leukocytes were obtained in pure state at the bottom of the centrifuge tube.

In the first experiments only qualitative determinations of the

¹ Jour. of Exper. Med., 1905, vii, 316; Opie and Barker, *ibid.*, 1907, ix, 207.

² Loc. cit.

³ Münch. med. Woch., 1907, liv, 3637; Deutsch. Arch. f. klin. Med., 1907, xci, 456.

⁴ Deutsch. Arch. f. klin. Med., 1907, cxi, 212.

⁵ Zeit. f. inner. Med., 1907, xxviii, 945.

proteolytic action of the leukocytes were made, and for this purpose the method described by Jochmann and Müller was adopted. A mixture of horse serum and bouillon, which was approximately the same as is recommended for the preparation of Löffler's blood serum, was coagulated in Petri dishes. Upon these plates were dropped equal quantities of the suspension of leukocytes and 0.85 per cent. sodium chloride solution, 0.1 and 0.2 per cent. sodium carbonate, 0.1 and 0.2 per cent. hydrochloric acid. One set of plates was usually incubated at 37° C., another set at 50° to 55° C. Jochmann and Müller state that proteolysis is much more active at the latter temperature than at 37° C. The presence of proteolysis was estimated by cupping and liquefaction of that portion of the blood serum covered by the drop of leukocytic suspension.

By this method it could be shown that the leukocytes of the blood of normal individuals contained definite enzymes, which acted in neutral, alkaline, and acid media and seemed to digest best at 55° C.

Washed pus was found to act in exactly the same manner as the leukocytes from the blood of normal individuals and of persons showing a marked leukocytosis. As it was impossible to obtain pus which was sterile, specimens were chosen which contained either pneumococci or streptococci, organisms that have little or no proteolytic action.

This simple method, which we first adopted, gave very striking evidence of the existence of proteolytic activity of the leukocytes, but afforded no definite information by which we could compare the action obtained in one experiment with that observed in another. It was therefore decided to estimate accurately the amount of digestion by the method of determining the nitrogen of the uncoagulable proteid. By this method it was found, as Opie has already stated, that the polymorphonuclear leukocytes digested best in an alkaline or neutral medium and very slightly in an acid medium.

The main object of these experiments was to study the proteolytic enzymes in the leukocytes in leukemia. It was possible to show in preliminary experiments that the leukocytes of normal blood, of the blood from cases showing a leukocytosis, and of pus, all contain a ferment which has the power of digesting proteids in neutral, alkaline, and acid media; and with pus, which is composed chiefly of polymor-

phonuclear leukocytes, this action takes place best in an alkaline or neutral solution.

It was possible to study the ferments in the leukocytes from the blood of a case of myelogenous leukemia, and it was found that the leukocytes in this case acted in much the same manner as the leukocytes of pus.

The patient was a man under the care of Dr. Da Costa at the Jefferson Hospital. To Dr. Da Costa we are greatly indebted for the opportunity of obtaining the material for study. The patient had suffered from myelogenous leukemia for years, and at the time that the blood was obtained had an enormous spleen filling most of the abdomen. The leukocytes were 274,000. A differential count showed that 89.6 per cent. of the cells were neutrophilic leukocytes and myelocytes.

There was perhaps more digestion in acid media than was obtained with pus alone, but otherwise, the washed leukocytes from the blood of this patient acted like the leukocytes of pus.

During this study we were most fortunate in having at the Pennsylvania Hospital, under the care of Dr. J. A. Scott, a typical case of acute lymphatic leukemia. The patient was a young man, a tailor by trade, and twenty-two years of age. When he was admitted on January 19, 1908, he had been acutely ill for five days with fever, headache, and abdominal pain, although for two or three weeks he had been feeling unwell and had suffered with what he supposed was "la grippe." On admission he showed some pallor, and had slight enlargement of the cervical and inguinal lymph nodes. There was a systolic murmur at the apex of the heart. The liver and spleen were not palpable. The illness progressed rapidly and was characterized by fever, varying from 101° to 104.4° , progressive anemia, a petechial eruption in the axillæ, repeated epistaxes, vomiting, and progressive weakness.

The examination of the blood showed a progressive anemia with an increasing leukocytosis.

The blood picture was characterized by the presence of typical large lymphocytes. These cells possessed large, round, oval, indented or somewhat irregular nuclei, staining rather palely with Wright's stain, and surrounded by a wide rim of protoplasm taking

a faint basophilic stain. Smears stained in Ehrlich's mixture showed no granules in the protoplasm of most of these cells, though a few definite neutrophilic myelocytes were seen.

Since the cells which formed the great bulk of the leukocytes (at death 77.7 per cent. of 414,000 leukocytes) of the blood were thought to be lymphocytes, it was suspected that they might have either no proteolytic action whatever, or if they did possess a ferment which digested proteid, that this ferment might act differently from the enzymes in the polymorphonuclear leukocytes and granular myelocytes.

The case terminated fatally, and immediately after death a trocar was inserted into the right side of the heart and blood withdrawn under aseptic conditions into 1.5 per cent. sodium citrate. In this way about 750 c.c. of blood were obtained. The mixture, which looked like yellow pus with a thin layer of blood at the bottom, was kept at 5° C. By centrifugalization pure leukocytes, free from red blood cells could be obtained.

The experiments with this material showed that these cells digested best in alkaline and neutral media, but were also quite active in the presence of acid.

When the figures in this experiment were compared with those obtained by the action of pus and of the leukocytes from the case of myelogenous leukemia, it could be seen that though the quantity of cells from the case of lymphatic leukemia, was three times as great as that in the other two experiments, digestion was not quite as good in neutral and alkaline media, but somewhat better comparatively in acid media. Since it could be shown, as will be seen later, that the amount of digestion varied with the quantity of cells employed, it may be suggested that though these large lymphocytes in lymphatic leukemia possess an enzyme which digests proteid in neutral and alkaline media, the ferment is not present in such great amounts as it is in the myelocytes and neutrophilic polymorphonuclear leukocytes.

The enzyme of the large lymphocytes seems to act better than the enzyme of the myelocytes and polymorphonuclear leukocytes when the reaction of the solution is acid.

Opie found that a powder made from the polymorphonuclear leukocytes retains for a long time that ferment of the fresh cells.

which digests in neutral and alkaline media, whereas the enzyme acting in acid media is not so stable and in the powder diminishes or disappears.

From the washed leukocytes obtained from this case of lymphatic leukemia a powder was prepared. Experiments performed with this powder gave much the same results as the experiments with fresh leukocytes. By using twice the quantity of powder the amount of digestion, when the experiment was done in the presence of 0.85 per cent. sodium chloride, was almost doubled and much increased when the solutions were made alkaline.

From these experiments it may be seen that the white cells in the blood in this case of so-called acute lymphatic leukemia possess a proteolytic enzyme which, though probably present in the cells in not so great amount, is still qualitatively the same as the proteolytic enzyme of the polymorphonuclear leukocytes and granular myelocytes.

Since the lymph nodes in this case were enlarged, and on microscopic examination were found to be made up almost exclusively of large cells similar to those found in the circulating blood, it was of interest to study the digestive ferments in these glands and to compare their action with that of normal lymph glands or of lymph glands from other pathological conditions.

As it was possible to obtain only one or two glands, this was not sufficient material to study the enzymotic activity quantitatively, but the experiments showed that the cells of these enlarged glands contained proteolytic enzymes acting best in an alkaline medium.

Lymph glands from other cases which showed slight hyperplasia were found to act on coagulated blood serum in an entirely different manner. Enlarged inguinal glands were obtained from a case of carcinoma of the penis. Microscopically the glands showed well-marked lymphoid hyperplasia with some proliferation of the large endothelioid cells. Digestion on coagulated serum plates took place only in the presence of acid.

Much the same result was obtained with an emulsion of the hyperplastic mesenteric lymph nodes removed at autopsy from a case of typhoid fever. Since the mesenteric lymph nodes in typhoid fever contain great numbers of large phagocytic cells, described by

Mallory and others as endothelioid cells, these lymph nodes seemed to offer excellent material for the comparison of the action of such cells with the large lymphocytes in lymphatic leukemia. Experiments demonstrated, however, that emulsions of glands containing great numbers of these large endothelioid cells act entirely differently from the large lymphocytes in the case of leukemia, and digest, as Opie has already shown, almost exclusively in the presence of acid.

Finally it was possible to study the action of the glands from a case of chronic lymphatic leukemia. For the material from this case of leukemia we are indebted to Dr. Funke and to Dr. William Pepper. The patient, during life, had an exceedingly high leukocyte count, the total number of white cells reaching at one time 1,000,000. Over 90 per cent. of the cells were small lymphocytes. At autopsy the lymph nodes were enormously enlarged, and on section showed a reticular meshwork, crowded almost exclusively with small lymphocytes, similar to those in the blood.

It could be shown that emulsions of the glands, as well as pieces of the glands themselves, failed to digest coagulated blood serum in any medium.

Though it was impossible to obtain lymphocytes from the circulating blood of a case of small-cell lymphatic leukemia, yet the last experiment seems to confirm very definitely the work of Jochmann and Müller, Stern and Eppenstein, and others, and shows that the small lymphocytes in the enlarged lymph nodes in that type of lymphatic leukemia, which is characterized by the presence of small lymphocytes in the blood stream, contain no proteolytic enzyme which is demonstrable by allowing the cells to come in contact with coagulated blood serum in the presence of neutral, alkaline, or acid solutions.

CONCLUSIONS. The leukocytes of the blood of normal individuals and of patients showing a marked polymorphonuclear leukocytosis contain enzymes capable of digesting coagulated blood serum in neutral, alkaline, or acid solutions.

The cells in pus that is composed principally of polymorphonuclear leukocytes and the leukocytes of the circulating blood in myelogenous leukemia contain similar proteolytic enzymes, which act best when the reaction is alkaline.

The leukocytes of the circulating blood and of the enlarged lymph nodes from a case of large cell, acute, lymphatic leukemia contain proteolytic enzymes that act qualitatively in much the same way as the leukocytes of pus and as the white corpuscles of the blood in myelogenous leukemia.

These large lymphocytes in acute lymphatic leukemia can be differentiated biologically from the small lymphocytes in chronic lymphatic leukemia which possess no proteolytic enzymes, and from the large, endothelioid cells of the hyperplastic lymph glands which are proteolytic only in the presence of acid.

These results seem to show that the large cells of the so-called acute lymphatic leukemia are not true lymphocytes, but are nearly related to the granular myelocytes, and should probably be considered as the forerunners to these cells.

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A Study of the Volume and Specific Gravity of Organs.¹

By SIDNEY L. OLSHO, M.D.

(From the Laboratories of the Jefferson Medical College Hospital.)

THE three linear dimensions in which the size of an organ examined at autopsy is recorded give to the reader a rather indefinite idea as to the actual size of the specimen. Viscera are irregular. The expressions "the organ is large," "fairly large," "voluminous," "larger than its fellow," "contracted," "splenic tumor," etc., are inaccurate and unscientific.

In order to determine and accurately register the size of any viscus, the following plan, employed at some institutions for registering the volume of the brain, should be adopted: Each organ as it is removed is submerged in a vessel filled with water to a level at which an overflow is provided. The water displaced overflows into a container

¹ Read by invitation.

graduated in cubic centimeters; the amount so obtained represents the volume or displacement of the organ in cubic centimeters. The organs are weighed in grams; the weight in grams, divided by the displacement in cubic centimeters, equals the specific gravity.

The heart is submerged opened or unopened. A "voluminous" emphysematous lung is pressed beneath the surface of the water by a rod thrust into the bronchus. It has been ascertained that no water enters the lungs—no bubbles appear because the contained air cannot be displaced. The liver, spleen, kidneys, or a tumor may be similarly measured. The record is thereby supplied with definite facts by which it is possible to appreciate the size of an emphysematous lung as compared with its atelectatic mate. The displacement in cubic centimeters constitutes a record, conveying an idea not obtainable from linear measurements even when the weight also is given.

Dr. L. Vervaeck, of Belgium, determined the specific gravity of organs and published his results in 1901. The method he used requires two weighings: one in and one out of water. His tabulations were based on the general clinical diagnosis of the case and not on the pathological condition observed in each organ. The specific gravity of the lungs was not determined.

In order to determine the practical value of the methods suggested I examined the organs from one hundred autopsies made at the Philadelphia Hospital.¹

HEART.—While the average specific gravity of hearts manifesting no evident abnormality was 1029, individual apparently normal hearts varied from 939 to 1152. It is not likely that healthy heart muscle varies in specific gravity to the extent indicated. Any marked deviation from the normal specific gravity, in a heart macroscopically normal, indicates that a histological study is necessary. In cloudy swelling—8 cases—the average specific gravity was found distinctly lowered, namely, to 1004. This conforms to observations made during the study, namely, that parenchymatous change lowers the specific gravity of the affected organ. In so-called chronic myocarditis, including general atrophy (20 cases), the average specific

¹ The notes of the cases studied may be found in the Philadelphia Hospital records, 1906, xxi.

gravity was 1008; lowest, 833; highest, 1088. Hypertrophied and dilated hearts (31 cases) yielded an average specific gravity of 1037; lowest, 969; highest, 1114. In simple hypertrophy (13 cases) the average specific gravity was 1032; lowest, 975; highest, 1128. Comparing hypertrophy with hypertrophy and dilatation combined, the previous observations seemed again to hold true. In dilatation, failure of nutrition and parenchymatous degeneration, the specific gravity was, as a rule, lower than in simple hypertrophy. The same general tendency obtains in chronic dilatation and fatty degeneration. Here with even greater parenchymatous change (12 cases) the average specific gravity is still lower, namely, 1025; lowest, 843; highest, 1214. Fatty infiltration presents a contrasting picture. Here with an intact musculature the specific gravity remained high, the average of 5 cases being 1061; lowest, 1000; highest, 1151.

KIDNEY.—The average specific gravity of the normal kidney (only 8 such available) was 1098; lowest, 1000; highest, 1190. In acute diffuse nephritis (20 kidneys) the average specific gravity was lowered to 1072.

In chronic parenchymatous nephritis (103 kidneys), commonly a diffuse lesion, the average specific gravity was further lowered, 1049. In one case the right kidney had a specific gravity of 1400. The left kidney was less granular; accordingly its specific gravity was only 1200. In chronic interstitial nephritis (58 kidneys) the average specific gravity, 1053, was higher than in chronic parenchymatous nephritis. As illustrating the influence of morbid processes in the displacement—size—of the organ, a comparison of the average volumes of the kidneys is interesting and suggestive.

Average volume, chronic interstitial nephritis (58 cases)	150 c.c.
Average volume, apparently normal kidneys (8 cases)	160 c.c.
Average volume, chronic parenchymatous nephritis (103 cases)	166 c.c.
Average volume, acute diffuse nephritis (20 cases)	185 c.c.
Average volume, acute diffuse nephritis and congestion (8 cases)	186 c.c.

While parenchymatous change seems to lower the specific gravity of the organ, the formation of fibrous tissue, on the other hand, raises it. This is perhaps best indicated by examination of the kidneys in chronic interstitial nephritis. Assuming that the kidneys of the smallest vol-

ume have undergone the most interstitial change—are most fibrous—the following comparisons may be made:

- 8 kidneys chronic interstitial nephritis, vol. 100 c.c. or less, av. sp. gr. 1257.
- 42 kidneys chronic interstitial nephritis,¹ vol. 100 to 200 c.c., av. sp. gr. 1025.
- 8 kidneys chronic interstitial nephritis,² vol. over 200 c.c. av. sp. gr. 994.

The organ becomes more dense because it contracts. The increased specific gravity contributed by fibrous-tissue formation is not demonstrable in every case, be it heart, liver, or kidney, because fibrous or interstitial processes are rarely dissociated from parenchymatous change. Where fibrosis is most marked, as in the group of small kidneys in chronic interstitial nephritis, the consequent increase of specific gravity is best illustrated.

LIVER.—In organs not the seat of any macroscopically evident lesion (11 cases) the average specific gravity was 1057; lowest, 1029; highest, 1088. The specific gravity is lowest in fatty infiltration of the liver (21 cases); average, 1028; lowest, 720; highest, 1098. In cloudy swelling, parenchymatous degeneration (12 cases), the average specific gravity was 1055; lowest, 1025; highest, 1086. In atrophic cirrhosis (7 cases) the average specific gravity was 1056; lowest, 1029; highest, 1069. In congestion amounting to red atrophy, average specific gravity was 1077; lowest, 973; highest, 1100.

SPLEEN.—Except in miliary tuberculosis the average specific gravity of the spleen is highest in chronic splenitis (28 cases), 1139, succeeded in order by the following: Acute splenitis (19 cases), 1110; congestion (17 cases), 1108; apparently normal (33 cases), 1043; acute suppurative splenitis (2 cases), 1040; amyloid (3 cases), 1027.

LUNGS.—As regards the lungs this method offers a more perfect mode of comparison of size of the two organs than can be obtained in any other way. In support of this statement the following cases may be cited:

CASE 38.³—Patient aged twenty-six years. Right lung: weight, 460; volume, 850; specific gravity, 541; chronic caseous tuberculosis. Left lung: weight, 780; volume, 770; specific gravity, 1013; chronic caseous tuberculosis and atelectasis of lower lobe.

¹ Probably less fibrous.

² Probably least fibrous.

³ Weight is given in grams and displacement, or volume, in cubic centimeters.

It is clear in this case that the right was the functioning lung; less weight, greater displacement, lower specific gravity.

CASE 39.—Patient, aged thirty-five years. Right lung: weight, 590; volume, 810; specific gravity, 728; edema and congestion; tuberculosis of lower lobe, hence higher specific gravity. Left lung: weight, 400; volume, 640; specific gravity, 625; edema and congestion.

CASE 53.—Patient, aged fifty-seven years. Right lung: weight, 560; volume, 580; specific gravity, 965; emphysema, congestion, healed tuberculosis. Left lung: weight, 190; volume, 180; specific gravity, 1055; atelectasis.

No description could give as adequate an idea of the conditions in this case as the figures quoted.

CASE 76.—Patient, aged twenty-four years. Right lung: weight, 460; volume, 670; specific gravity, 686; acute miliary tuberculosis. Left lung: weight, 460; volume, 610; specific gravity, 754; acute miliary tuberculosis.

The two lungs weighed the same; the right lung was larger; the left should have weighed less; the specific gravity of the left was the higher. From the figures alone it is proper to conclude that the left lung was the more involved.

CASE 88.—Patient, aged forty-six years. Right lung: weight, 660; volume, 1200; specific gravity, 500; emphysema. Left lung: weight, 940; volume, 1300; specific gravity, 723; lobar pneumonia involving part of upper lobe; the remainder of the organ emphysematous.

CASE 108.—Patient, aged fifty years. Right lung: weight, 540; volume, 1150; specific gravity, 469; the organ apparently normal, crepitating throughout. Left lung: weight, 1790; volume, 1780; specific gravity, 1005; lobar pneumonia; nowhere crepitant.

These few cases are sufficient to illustrate what is already known, namely, that conditions like fibrosis, atelectasis, and pneumonia increase the specific gravity of the lung. Comparison of weights, volumes, and specific gravity of the two lungs gives an approximate idea of the amount of functioning tissue present in each.

CONCLUSIONS.—A statistical study of the organs of 100 consecutive autopsies seems to indicate:

1. Parenchymatous degeneration lowers the specific gravity of organs proportionately to the degree of parenchymatous change.

2. Fibrotic change, while diminishing the volume of the organ, also raises its specific gravity proportionately to the amount of fibrosis.

3. Although useful in systematic studies of all organs, the specific gravity records are most striking in pulmonary affections.

March 12, 1908.

**Note on the Occurrence of a Ciliate (*Opalinopsis nucleolobata*, n. s.)
in the Liver of a Mammal (*Canis latrans*).**

BY ALLEN J. SMITH, M.D., AND HERBERT FOX, M.D.

(From the McManes Laboratory of Pathology of the University of Pennsylvania,
and the Laboratory of Comparative Pathology of the Philadelphia
Zoölogical Gardens.)

THE following record seems to the writers worthy of publication, because, so far as they are aware, there is but one other case mentioned in medical literature in which a ciliate was noted as a parasite of the mammalian liver, and because, provided the identification of the organism here dealt with as of the family of *Opalinidae* be correct, it is the first time in which it has been found that any member of this family has been parasitic in a mammal, the various species being known only as parasites of worms and other invertebrates, and of frogs and toads.

In stained sections¹ of the liver of a prairie wolf, *canis latrans*,² these ciliates were discovered in large numbers. Unfortunately, the writers are forced to depend entirely upon preserved material, as no idea of their occurrence was had prior to their discovery in the finished histological preparations. This fact materially limits the study, as much of the examination of such specimens is necessarily or preferably to be carried out upon the fresh and living protozoa.

The coyote had been in the Zoölogical Gardens for about two years, but was a poor inbred specimen, was never on exhibition, and was ordered killed on April 3, 1907. The autopsy was performed very shortly after death and the material for microscopic examina-

¹ University of Pennsylvania Path. Hist., No. 2199.

² Philadelphia Zoölogical Garden Laboratory, 1048.

tion at once fixed in formaldehyde solution. There existed a hypostatic congestion of both lungs, and a slight grade of general visceral fibrosis, an interstitial nephritis being especially developed. In various places in the liver there were indefinitely outlined areas, varying from 15 to 30 millimeters in diameter, which upon the surface of the organ formed slightly convex prominences, somewhat paler than the rest of the hepatic substance and a trifle softer, superficially suggesting the appearances of small abscesses. In section, these swelled out above the rest of the cut surface and seemed to consist of irregularly shaped liver lobules with blotches of a brownish or yellowish-brown pigment in and among them. The alimentary canal was grossly normal, and no part was saved for microscopic examination.

In the histological preparations of the liver there were no notable alterations of the general structure (beyond a slight perilobular cirrhosis) save in connection with these areas. In the latter the blood-vessels were irregularly dilated, at places the tissue approaching the appearance of an angioma, this affecting especially the intralobular capillaries here and there, and there was a small amount of hemic pigmentary deposit. Here and there in these nodes were patches of hepatic cells presenting a fine vacuolation, converting the protoplasm into a fine reticulum, but without involvement or change in the nuclei (probably edema of the cells rather than fatty change). Between the liver cells and often definitely within the dilated blood channels of these nodes the ciliates were found, sometimes widely separated from each other or again in numbers in the tissue of a single field of the microscope. In the vicinity of the infusoria, and about them, there was often a minor infiltration of the tissue by polynuclear and rather large mononuclear leukocytes; but there was in no instance any definite encapsulation of the organisms. The gall-ducts showed no invasion by the parasites, the epithelial lining of these channels being quite normal, although in places there appeared a slight increase of the peribiliary connective tissue in the sections examined.

The organisms are spheroid to short ovoid in outline, the largest ones attaining a long diameter of 0.035 mm. (exclusive of the ciliary border); the smaller recognized individuals often being less than half of this measurement. In case of many of the parasites no ciliated border can be made out, this being particularly true of small

examples and individuals about which the cellular elements of the organ (hepatic cells, red blood corpuscles and leukocytes) are closely packed. None are seen with partial ciliation. Typically, the organisms are holotrichous, the entire border being thickly and uniformly set with rather coarse, more or less matted cilia, without any appreciable local differentiation at any position. The existence of cuticular striation is uncertain; it is not in the least evident in the great majority of examples, but there were very faint suggestions of a longitudinal striation noted in a few of the parasites (this point would have been clearer, doubtless, in the fresh state, and must be held in doubt). Beneath the ciliary surface there is a sharply defined and fairly thick, somewhat refractile cell wall taking a slight eosin tint in the hematoxylin and eosin preparations. The cytoplasm, staining in the same combination a faint eosin hue, is finely granular and without clear differentiation into endosarc and ectosarc. No trace of a mouth, pharyngeal depression, anal orifice, or vacuoles are recognizable, and the substance is entirely free from the coarse granules which are common as ingesta in most ciliates. The nucleus is very variable in its appearance. No micronuclei are recognized in examination of hundreds of examples (but the known difficulty of demonstrating the micronucleus of infusoria save in the fresh unstained specimen should be recalled and the failure to detect this body in our material is not to be regarded as certain evidence of its absence). Ordinarily the nucleus is relatively large, often occupying, especially in small individuals, quite as much of the cell as does the nucleus of a small lymphocyte. In the smaller specimens the nucleus is apt to be simple, round or oval, taking on the hematoxylin hue deeply. In the larger ones the nucleus is a large multilobulate mass, the lobules commonly clumped together so that their relations are not clear. A few instances in which the nucleus shows a circular or horseshoe shape, with the nodules appearing as swellings upon the band, and a few others with elongated, irregularly band-like nuclei, indicate to the minds of the writers that the parasites are not multinucleate, as in the genus *Opalina*, but that the nuclear lobulations all belong to one nuclear mass. Either as a stage of vital existence or as the result of regressive changes (more probably the former), nuclei are to be found broken into thick cord-like fragments,

reminding one of coarse chromatic elements in mitosis, but certainly not of the appearance of mitotic figures as found in infusoria (such nuclei, too, are clearly to be regarded as macronuclei, whereas mitosis in the infusoria involves the micronucleus). Division of the nucleus is apparently a direct one, and the division of the cell takes place by a simple hour-glass constriction following the nuclear division. The writers have also met with examples in which there are clearly separate nuclei or parts of nuclei, but after careful consideration are disposed to believe the picture artificial, due to section of the nucleus in such manner that some of the lobular divisions are separated from the major mass. So, too, we would interpret the occasional examples of non-nucleated ciliates in the sections on the assumption that they are parts of large examples which have been separated by the plane of section from the larger part of the cell which bears the nucleus. There were met relatively few examples enclosed in a double wall, but such may be accepted as instances of encystment of the parasites. The large number of specimens about which the ciliary border cannot be detected are in part evidently the result of obscuration of the ciliæ by the close packing of other elements about the infusoria, and in part probably to poor fixation (this idea is upheld by the fact that the ciliæ are by far best seen in sections made from thin blocks of tissue early selected and fixed for histological study, while the ciliation is less frequent and more poorly shown in material later taken from larger masses of the liver coarsely preserved); doubtless, too, in some instances the ciliæ have been retracted prior to encystment of the parasites.

It seems very improbable that in material thus prepared there could have been lost with uniformity all appearance of mouth, pharynx, contractile vacuoles, and similar organelles; and the writers, therefore feel justified in referring the organisms to the family *Opalinina*, which is characterized by the absence of these parts. The absence of coarse fragments from the cell, as are commonly ingested by infusoria, is confirmatory of this view; the organisms probably obtaining their nutrition and carrying on excretion entirely through the cuticle. Primarily, from the occasional appearance of separateness of the nuclei above referred to, and from the apparent absence of micronuclei, the writers believed the specimen to belong to the genus

Opalina; but more careful consideration of certain nuclei, and in instances where, probably from artefact, the nucleus is being extruded from the organism, has reversed this view and justified the idea of a single multilobulate nucleus. The characteristics determined coincide with those of the genus *Opalinopsis*, Foettinger,¹ 1881. It is to be noted, however, that none of our specimens exhibit the reticulate type of nucleus (nuclear substance as an anastomosing network occupying much of the cell) which Foettinger described in the species upon which the genus was based (*Opalinopsis sepiolæ*, *Opalinopsis elegans*, and *Opalinopsis octopi*), which were encountered in the liver of several sepiolæ and of an octopus. In the living animals, too, Foettinger was able to make out the existence of a number of small non-contractile vacuoles. If such existed in our material in the living state they may well have been so reduced in fixation as to have become inapparent. Careful comparison of material with Foettinger's text and description makes it certain that the specimens in hand are not specifically identical with any of the known species of opalinopsis, and the writers would offer the examples here described as a new species, for which they would suggest the name *Opalinopsis nucleolobata*. The determined features of the species in synopsis are: *Spherical or short ovate holotrichous infusoria, without oral or anal orifices, without pharyngeal depression; with no differentiation of the rather shaggy ciliæ at any position; apparently without any form of vacuole; micronucleus doubtful or absent; nucleus large, single, multilobate (simple in early forms), occasionally in band and beaded form and sometimes showing as coarse cord-like fragments; no definite differentiation between ectoplasm and endoplasm; size up to 0.035 mm. in greater diameter; cuticular striations uncertain.*

The only other instance known to either of the writers at present in which a ciliate has been found as a parasite in the mammalian liver is recorded in *Transactions of the Pathological Society of London*, 1899; *Paramœcium coli*, an occasional parasite of the human large intestine, having been met in the liver of a man who had died of malignant disease of the stomach. The living parasites were found in the cheesy contents of numerous small cysts connected with the

¹ Arch. de Biologie, 1881, ii, 354.

gall-ducts. Apparently the parasites had reached the liver by way of the common bile duct from the intestine, setting up an irritation which occasioned the formation of the cysts.

In the case above detailed it is quite uncertain how the parasites gained access to the liver of the coyote and what was the source. Opalinopsis as described by Foettinger is thus far known as a parasite of the liver in sepiola and octopus; perhaps the species encountered has its natural habitat also in the liver of some low type, and by preference sought this organ after being introduced into the economy by the alimentary tract. Occasionally canine animals devour frogs and toads or even lower forms of animal life; and, at least, this may be thought of as a probable source until the organism is discovered in its proper host.

March 12, 1908.

Comparative Study of Noma.

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NOMA is of interest on account of its comparative rarity and of the diverse opinions expressed by different observers in regard to its etiology. The condition becomes of added interest when found in the lower animals, and especially in a wild animal.

Various causes for this disease have been advanced. The older writers thought that the condition was due to a thrombosis or to trophic changes. These theories have been dismissed because the disease is not confined to the course of any one bloodvessel or nerve. The probability of its being of an infectious nature is now looked upon as certain.

The findings of the various observers may be roughly grouped under various heads. Thus:

(a) Moser¹ in three cases found protozoa.

(b) Walsh² and Levi and Sailor³ found, culturally, the *Bacillus diphtheriæ*; Goepf, ⁴ the *Bacillus diphtheriæ* associated with the

Staphylococcus albus and the *Streptococcus pyogenes*; Petruschky,⁵ the *Bacillus diphtheriæ* accompanied by vibrios and spirilla; and Bishop,⁶ the *Bacillus diphtheriæ* both culturally and in the tissues.

(c) Ranke⁷ found, both culturally and histologically, streptococci resembling those already described by Koch which occur in progressive tissue necrosis of field mice.

(d) Guizette⁸ and Babés and Zambilovici⁹ found fusiform-shaped bacilli both culturally and in the tissues, while Rosenberger¹⁰ and Weaver and Tunncliffe¹¹ obtained in smears the *Bacillus fusiformis* accompanied by a spirillum similar to the organisms which Vincent¹² had previously noted in the necrotic tissues of hospital gangrene, a disease analogous to noma.

We now come to a large group of observers, many of recent date, whose findings, while similar in many respects, differ in the minuter details.

This group is composed of those who have found histologically, and in some cases culturally, bacillary microorganisms forming thread or filament-like processes, and in some cases spirillum forms.

As early as 1883, Lingard and Batt¹³ described bacilli found in rapidly advancing necrotic disease in the mouths of cattle.

Later, Lingard¹⁴ found, in necrosis of the mouth in man, monkeys, and calves, and in gangrenous pneumonia in horses, a bacillus forming long threads.

Grawitz¹⁵ found bacilli forming long threads which were Gram positive.

Bartels¹⁶ observed bacilli, threads, and cocci.

Schimmelbusch¹⁷ noted rounded bacilli forming long threads in the central part of the gangrenous area.

Foote¹⁸ reported the presence of long bacilli joining end to end in long strings.

Blumer and McFarlane¹⁹ demonstrated a leptothrix forming short bacilli and long threads.

Perthes,²⁰ Krahn,²¹ and Brüning²² observed bacilli forming threads and spirillum forms (Perthes calling this organism a streptothrix).

Schmorl²³ found long thread-like organisms resembling the *Bacillus necroseos*.

Finally, Jensen²⁴ and others, viz., Bang, Kitt, McFadyean, etc.,

have noted the *Bacillus necroseos* in this condition in lower animals such as cattle, sheep, kangaroos, monkeys, and even birds.

Herrman,²⁵ in summing up the literature on noma, endeavors to simplify the different findings noted by advancing the theory that the spirillum of Vincent is simply a stage of development of the *Bacillus fusiformis*, and that this latter organism and the streptothrix of Perthes are identical with the *Spirillum sputigenum* and the *Spirochete dentium* which Miller found in the normal mouth as saprophytes. Herman further states that he considers the *Spirochete dentium* as only a stage of development of the *Spirillum sputigenum*, thus claiming (as previously held by Krahn) that noma is probably caused by the saprophyte *Spirillum sputigenum* assuming under favorable conditions a parasitic role. He would call this organism the *Spirochete* of necrosis. He still further states that he considers the *Bacillus necroseos* of lower animals to be closely related to this *Spirochete necroseos*.

All the observers who have studied the necrotic tissues histologically, have noted that in the superficial areas of advanced necrosis there was a polymicrobial infection, while the nearer they approached the healthy tissue the fewer became the microorganisms until along the zone between the healthy and necrotic tissues each found the particular microorganisms which he described in almost pure culture.

Notable among the reports of the various observers is that of Perthes, who found histologically along the line of demarcation rod- and spindle-shaped microorganisms forming long thread or filament-like processes and spirillum forms. The nearer the examination approached the healthy tissue the less frequently were found the threads, until along the immediate border of the necrotic tissue and growing into the healthy structures were demonstrated fine spirillum-like processes in great numbers. These rods, threads, and spirillum forms he considers to be different stages of development of a single microorganism, which he thinks is the same as the bacillary and thread-like organisms found by others in man and animals, the spirillum forms probably remaining hidden on account of their poor staining properties. Krahn and Brüning have corroborated his findings, Brüning further noting the penetration of the bloodvessel walls in the necrotic areas by these bacilli.

These bacilli, fibers, and spirillum forms were found to be Gram negative.

In regard to the clinical side of noma, it has been noted by several observers that it started as an ulcerative stomatitis; in fact, being an advanced stage of this condition or a gangrenous stomatitis. The primary lesion is generally a gingivitis beginning about the teeth and advancing to the grosser lesion of noma with all its characteristics.

The case which we would present to-night is one of noma or gangrenous stomatitis occurring in a wild animal, a Rhesus Macaque monkey at the Zoological Gardens of Philadelphia.

The subject being a wild animal, there is naturally not much of a clinical history.

This monkey had not been sent to the exhibition cages, but was still held in quarantine for further observation, when attention was called to a purplish discoloration limited to a small area upon the lower lip. This looked exactly like an ordinary bruise such as the animal might receive in handling or in the small cage. In twenty-four hours this area had increased greatly in size, practically involving the whole lower lip, which was greatly swollen, bluish black in color, and showed a distinct line of demarcation. Upon closer examination the mucous membrane of the gum was found to be ulcerated, the ulceration extending to the inferior maxillary bone, which was later found to be eroded. At the labiogingival junction there was a purulent exudate upon the mucous membrane. Before the animal was killed smears were taken from this exudate and the diseased areas, and cultures were made on all the ordinary culture media.

Postmortem examination of the animal showed the internal organs to be in a normal well-nourished condition, with the exception of a small area of verminous pneumonia of the upper lobe of the left lung. Further examination of the lip and mouth showed the diseased area to be a necrotic process, sharply circumscribed on the lower lip and adjacent soft parts, that extended from nearly the angles of the mouth toward the chin for about 1 cm. externally, while internally the whole mucous membrane of the lower lip and gum to the border of the incisor teeth was involved.

Section through this area showed the adjacent part of the lower maxillary bone to be eroded, and the softer tissues adjacent to the pale

necrotic area greatly reddened from congestion. Sections through the whole area were taken for histological study, with the following findings:

HISTOLOGICAL EXAMINATION. Sections stained with eosin-hematoxylin. The diseased area shows a gangrenous process involving all the tissues and extending from the skin surface of the lip to the gingival border.

All the cellular structures of the part show the same degree of degeneration (that is, loss of all protoplasmic and nuclear-staining characteristics) extending to an irregular but sharply circumscribed line of demarcation. Beyond this line of demarcation toward the healthy tissue there is a slight zone of inflammatory reaction, consisting chiefly of polymorphonuclear leukocytes and engorged and dilated capillary and larger bloodvessels. Beyond the reaction zone the normal healthy structures of the part are found.

Sections stained especially for bacteria show in the superficial parts, that is, the exposed part grossly, an infiltration with numerous forms of bacteria, in which cocci predominate and which are probably the ordinary mouth organisms. These infiltrating bacteria become less and less prominent as the deeper parts of the necrotic area are examined, and give way to bacillary and thread-like organisms which increase in number until a felt-like network of them is found near the line of demarcation. In this felt-like formation the bacillary organisms can be seen varying in thickness and length with the production of rods and threads extending in all directions.

Along the line of demarcation these organisms are less in number than in the felt-like area, but are still numerous and can be better studied here.

Here they vary in size and thickness, the more delicate threads predominating. Here also may be seen these organisms, some with angular bendings and some with irregularly stained bands, which seem to form small filaments which push between the cells of the part and the infiltrating leukocytes. These grow less in number and more delicate as they proceed toward the healthy tissue.

Sections stained by Gram's method are negative for these special bacillary and thread-like forms. A section stained by a special method, as recommended by Jensen for the *Bacillus necroscus*, which has been

found by others in spontaneous gangrenous processes in animals, failed to show the presence of these microorganisms.

Sections were also stained with Weigert's elastic stain to exclude possible confusion between the remnants of elastic tissue normal to the part and these threads, and showed that these threads were foreign to the tissue.

BACTERIOLOGICAL EXAMINATIONS. The smears which were made from the exudate and the superficial necrotic areas were stained with Loeffler's alkaline methylene blue, and showed a great increase in the number of spiral microorganisms which were of various lengths and number of twists, some being robust and some very delicate resembling the spirillum of Vincent. In these spreads fusiform bacilli were also found, some of which were slightly curved; others showed irregular staining. Unfortunately no wet specimens were examined for motility.

The cultures which were grown aërobically showed the presence of streptococci and staphylococci, but no fusiform bacilli nor spirilla, particular attention being given to the growth of organisms in the water of condensation of the agar-agar and blood serum tubes.

No anaërobic cultures were made.

As a result of these findings and in view of the fact that Grenet²⁶ and Weaver and Tunnicliff had found the *Bacillus fusiformis* and spirillum of Vincent in normal mouths, and Angelici²⁷ had found the *Bacillus fusiformis* normally in man and lower animals, the question arose in regard to the frequency of spiral microorganisms and fusiform bacilli in the mouths of normal monkeys. To determine this smears were made from the mouths of 25 monkeys and examined for these organisms.

Of our 25 stained smears, 12 showed the presence of spiral microorganisms in very limited number; 18 showed the presence of fusiform bacilli also in limited number, and 11 showed both spiral and fusiform bacilli present in the same smear. In only 2 of the smears showing the spirals and fusiform bacilli in symbiosis did the spiral organism resemble the spirillum of Vincent. The monkey from which one of the latter smears was taken showed clinically an erosion of the mucous membrane of the lower lip, the monkey of the other smear being apparently normal. Wet specimens were made from 11 of these monkeys, but only in one instance was a spirillum found.

SUMMARY. 1. This is a case of noma occurring in a wild animal and showing the anatomical and clinical characteristics of the disease.

2. A fusiform bacillus and a spiral microorganism resembling Vincent's spirillum are found in the exudate and scrapings of the diseased area, there being also a great increase in spiral microorganisms.

3. The histological examination showed the presence of rod- and thread-like organisms similar to those described by Perthes, Brüning, and others in the diseased area, being especially located in the area of advancing disease.

4. A spiral organism resembling Vincent's spirillum in conjunction with a fusiform bacillus is found in the normal monkey's mouth.

5. Fusiform-shaped bacilli are frequently present in small numbers in the mouths of normal monkeys.

CONCLUSION. In conclusion we can make no claim that the organisms present are the cause of this condition of noma because of our failure to isolate and cultivate them, but would call attention to the fact that our findings are similar to those reported in a corresponding condition in human beings by others who have advanced the theory that they are the cause of noma or other gangrenous processes of the mouth.

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Address of the President.¹

By JOSEPH MCFARLAND, M.D.

As the time for our annual meeting drew nearer and nearer, marking the close of another year of our work as a society, I became more and more perplexed as to the best use to be made of that part of the evening set aside for the "President's Address." One after another a variety of subjects suggested themselves, but were cast aside with some impatience, for there was slowly but surely taking possession of my mind the conviction that upon this occasion the President of the Society ought to have a confidential talk with the members, reviewing the year's work critically, commenting favorably where conditions were good, adversely where otherwise, and forecasting the probabilities for the future.

I seemed to find stored away in the dark corners of memory's closet back records of certain addresses of my predecessors who pursued this same course, but lest I should become a repeater or an imitator I carefully avoided looking up any of their expressions. If, therefore, my remarks this evening follow the same general direction as theirs, it must be put down as a matter of some scientific interest that the same psychological necessity being experienced we were driven to the same general plan of action.

¹ Delivered at the Annual Meeting of the Pathological Society of Philadelphia, October 22, 1908.

Since our Society was formed, new conditions have arisen to hamper its success. When it was new there was probably no other association of medical men by whom medical subjects were treated chiefly from the scientific, *i. e.*, biological, point of view. In recent years, however, there have been formed the American Society of Bacteriologists, the Association of American Pathologists and Bacteriologists, the Laboratory Section of the American Public Health Association, and the Section on Pathology of the American Medical Association, in all of which bodies contributions are limited to technical papers in which the biological problems are dealt with to the exclusion of, or at the expense of, their immediate practical application.

In addition to these we have the Association of American Physicians with an omnivorous appetite and vast assimilative powers for papers upon the experimental investigation of all kinds of medical problems. All of these societies injure us, more or less directly, by drawing away many contributions that would otherwise fall to our portion, and indirectly by diminishing our relative importance. I might even include the Society of Normal and Pathological Physiology as aiding in this general disaffection.

But I must not make myself misunderstood. Though all of these organizations act more or less injuriously upon us as a Society, their formation is, in itself, of far-reaching good by drawing together capable workers from all parts of the country to read and discuss their best work and so promote national medical improvement. We are simply the small minority that always experiences some injustice when a great good is accomplished.

But, notwithstanding the stress of these losses, when one takes our program for a year and refreshes his recollection of what has really been accomplished, he cannot but be astonished at the extent and scope of our contributions and the variety of our exhibition material.

During the year beginning with the last annual election, October 10, 1907, and ending tonight, we have held fifteen meetings. There was no meeting November 28, Thanksgiving Day, none December 26, and none in June, July, August, September, or in the first two weeks of October, the late convention of our Society this autumn being caused by the meetings of the International Conference and Congress on Tuberculosis. The average attendance at these meetings was

small, but at such as might be designated "special occasions" the room overflowed.

During the year thirty-four papers were read. I suppose one would be justified in supposing that a critical survey of these papers would indicate the subjects of particular interest to the Philadelphians, but allowances must be made for the presentation of much of the best work of Philadelphians before other and larger societies, and for the almost universal tendency to present some rare or curious thing rather than that of particular interest to the writer. This applies both to the papers read and to the specimens presented.

Some of the papers were devoted to the collection of known facts—useful summaries of the literature, and were usually presented by invitation. Some were descriptions and interpretations of things seen at the bed-side, at the autopsy table, or in the microscope. Some are records of interesting and ingenious experiments; and others are elaborate monographs upon special subjects. I have found it difficult to classify the papers otherwise than by subjects, and I may have done it badly. Alphabetically arranged they include the following:

Bacteriology and its Collaterals.

Diagnosis of Diphtheria by Smears.

Significance of Tubercle Bacilli in the Feces.

Actual Significance of Tubercle Bacilli in the Feces.

Bacterial Vaccines of Staphylococcic Strains; a Technique for their Preparation.

A Study of the Colon Aërogenes Group of Bacteria.

New and Improved Method for the Presumptive Test for *Bacillus coli communis*.

Bile Ducts.

Cysts of the Common Bile-duct.

Blood.

Study of the Hemopoietic Organs in Diphtheria and Tuberculosis.

Acute Lymphopenic Lymphatic Leukemia.

Proteolytic Ferments in a Case of Acute Leukemia.

Clinical.

Venous Pulse.

Observations on Urinary Tube Casts.

Experimental.

Liver Necroses from the Intravenous Injection of Ether during Life.

Volume and Specific Gravity of Organs removed at Autopsy.

Tissue Transplantation into Other Species.

The Coördination of Gastric and Intestinal Digestion by the Action of the Pyloric Sphincter.

Immunity.

Phagocytosis in Diphtheria.

Leukocyte Counts before and after the Administration of Antitoxin.

Kidneys—Nephritis.

Congenital Nephritis.

Nerves.

Interpretation of Appearances seen in a Peripheral Nerve.

Parasites.

Ciliated Organisms in the Liver of a Prairie Wolf.

Parasitic Nodular Conjunctivitis.

Cysticercus Tenuicollis.

Spleen.

Splenomegaly.

Syphilis.

The Present Status of the Spirocheta pallida (Treponema pallidum).

Immunity in Syphilis.

Tumors.

Production of Experimental Deciduoma.

Carcinoma of the Esophagus.

Metastatic Squamous Epithelioma of the Esophagus.

Sarcoma of the Eyelid.

Report of a Case of Metastatic Carcinoma of the Lung.

Brief Report of a Growth of the Testicle Resembling Sarcoma, with Metastases to the Lung.

Vessels.

Peri-arteritis Nodosa.

It appeals to me as remarkable and regrettable that there should have been no paper upon any subject related to chemistry, as the future of Pathology is supposed to lie hidden behind the chemical horizon. It is also striking that there should be complete silence upon subjects relating to the central nervous system, the cardiovascular system (we had only Longcope's paper upon "Peri-arteritis Nodosa"), the digestive system (we had only Lavenson's paper upon "Cysts of the Common Bile Duct"), the respiratory system, the bones and joints, the muscles, and the skin. Truly this is the passing of morbid anatomy! Has all been said upon these subjects, or do we fear that they are too familiar to be made the subjects of further communications? Anyone who has my experiences with recent graduates and young hospital residents, knows how little they know about hearts; and anyone who feels as uncertain as I do when I try to interpret what I see on the gastric mucosa, knows how much more he needs to know about the stomach.

But some of the omissions in writing have been made good by demonstration, and the table that was spread before us during the year groaned with good things enough to satisfy the most epicurean appetite for variety. Until I reviewed these lists, I had no idea, myself, how fertile was the soil we cultivate or how rich the harvest of our specimens.

Let us examine the lists and see what has been shown to us in a year. In all there were fifty-four specimens, and include the following:

Aneurysms:

One of the aorta.

Apparatus:

A new coverslip holder.

A guide for the use of the Maltwood's finder.

Arthritis:

Hypertrophic arthritis.

Bacteria:

Treponema pallidum.

A hitherto undescribed organism.

Bile Ducts:

Cysts of the common bile ducts.

Bladder:

Necrotic cystitis, with calcareous deposits.

Blood:

Specimens of leukemia.

Nucleated red crisis in leukemia.

Bones:

Skulls showing inadequate cranial repair.

Brain:

Abscess of the left ventricle.

Diffuse meningitis simulating abscess.

Cerebrospinal meningitis.

Calculi:

A tonsillolith.

Heart:

Aortic stenosis.

Aneurysm of the sinus of valsalva

Malignant endocarditis.

Endocarditis.

Bicuspid aortic valve.

Intestine:

Perforated ulcer.

Kidney:

Papillary cysts.

A pair of interesting kidneys.

Liver:

Cirrhosis.

Leukemia.

Angioma.

Fatty infiltration.

Necrosis after the intravenous injection of ether.

Metaplasia of the epithelial lining of the gall-bladder.

Section of gall-bladder containing a mass of lymphoid tissue resembling Peyer's patch.

Lung:

Infarction.

Cancer: Secondary to mamma.

Cancer: Secondary to mamma.

Rupture of subdiaphragmatic abscess into the lung.

Malformations:

Situs inversus vicerum sine dextrocardia.

Syndactylism.

Spina bifida.

Imperforate urethra.

Meckel's diverticulum.

Parasites:

Cysticercus tenuicollis.

Eustrongylus gigas.

Strongyloides intestinalis.

Strongyloides intestinalis.

Ciliated organism in the liver of a prairie wolf.

Parasitic organisms in nodular conjunctivitis.

Photographs:

Lumière photographs of various skin lesions.

Spleen:

Liver and spleen from leukemia.

Splenomegaly.

Stomach:

Carcinoma.

Squamous-cell carcinoma.

Syphilis:

Treponema pallidum.

Tuberculosis:

Of retroperitoneal glands.

Of seminal vesicles.

Tumors:

Specimen showing dissemination of a uterine carcinoma through the retroperitoneal glands and thoracic duct.

Carcinoma of stomach.

Squamous-cell carcinoma of stomach.

Adrenal "rests"—one developing into a hypernephroma.

Syncytioma.

Angioma of the liver.

Malignant struma: Microscopic specimen from Prof. Langhans.

Cancer of lung.

Cancer of lung.

Syncytioma of the testes.

Giant-cell sarcoma of the forearm.

Sarcoma of the glands of the esophagus.

Sarcoma of the eyelid.

Vessels:

Aneurysms of the aorta.

Peri-arteritis nodosa.

It seems to me that this list is one upon which we can congratulate ourselves. It is very interesting, and I think important to notice in passing that these specimens were presented by no less than thirty-six different individuals; and when to this fact is added that sixty-five *different persons*, or 22 per cent. of our entire membership, participated in the meetings of the year, otherwise than by discussion, I think it is very gratifying and shows that interest in subjects related to pathology is not confined to a few men who happen to teach the specialty, but extends to many others whose chief activities are in hospitals and dispensaries.

Considerable profit and enjoyment resulted from the excellent plan of the Business Committee, upon the efficiency of whose work I cannot too favorably comment, in arranging the symposium upon "Syphilis," at which Drs. Flexner and Torry gave a beautiful demonstration of living spirochetæ; and the symposium upon the "Para-

thyroids," at which we were profitably entertained by Professor Halsted and Dr. W. G. MacCallum.

We all remember with interest the address of Professor R. M. Pearce, at the semi-annual Conversational Meeting upon "The Theory of Chemical Correlation as Applied to the Pathology of the Kidney," and the two other addresses by invited guests, Professor W. B. Cannon, of Harvard, upon "The Coördination of Gastric and Intestinal Digestion by the Action of the Pyloric Sphincter," and of our own Dr. Donaldson upon the "Interpretation of Appearances Seen in a Peripheral Nerve."

I am pleased to announce that our membership numbers three hundred and two; during the year we lost six members but gained nineteen, so that we are richer than before.

It is with profound regret that I recall our loss in the death of Dr. J. Dutton Steele. The patience and perseverance with which he applied himself to the study of those problems that interested him should be an inspiration to every member of our Society.

We are about to embark upon another year's endeavor. With this excellent record behind us, we can confidently hope for a prosperous future.

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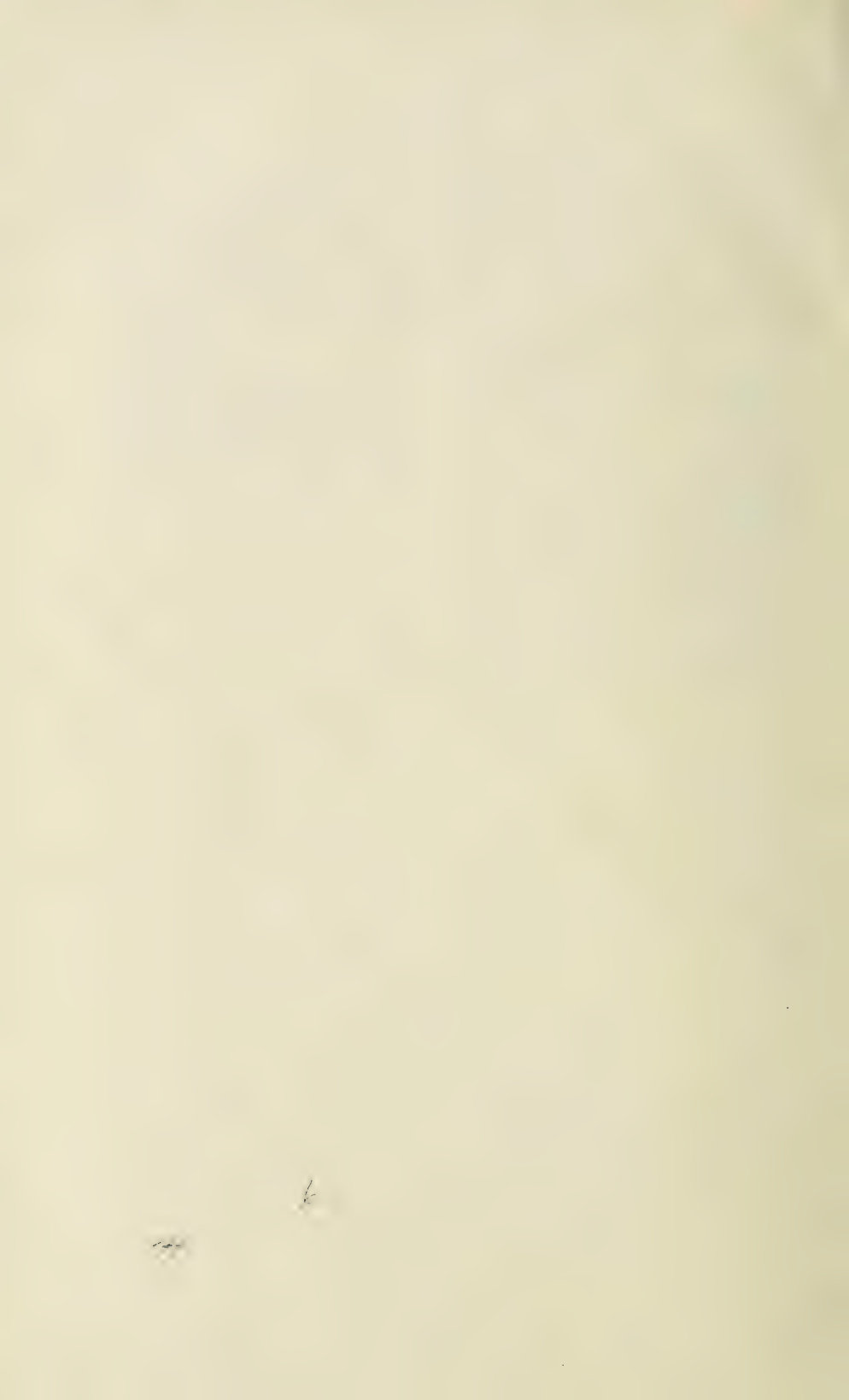
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